Modulation of Adult Hippocampal Neurogenesis in Laboratory- and Wild Mice

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ZUSAMMENFASSUNG

Die Bildung von Neuronen aus Stamm- oder Vorläuferzellen im adulten Gehirn, die sogenannte adulte Neurogenese, konnte im Gehirn von vielen Säugetieren, darunter auch beim Menschen, in mindestens zwei Regionen nachgewiesen werden. In der subventrikulären Zone des olfaktorischen Systems werden Neurone gebildet, welche während ihrer Reifung in Richtung des olfaktorischen Bulbus wandern, sich dort in das bestehende neuronale Netzwerk eingliedern und funktionell zur Unterscheidung von Gerüchen beitragen. Im Hippocampus findet die Neubildung von glutamatergen Neuronen in der subgranulären Zone des Gyrus dentatus statt. Die funktionelle Aufgabe dieser jungen Neurone ist umstritten. Ein funktioneller Beitrag zu Hippocampus-abhängigen Aufgaben scheint wahrscheinlich.

Zahlreiche interne und externe Einflüsse wie Stress, physische Aktivität, stimulusreiche Umgebung, Wachstumsfaktoren, sozialer Kontakt usw. wirken sich positiv oder negativ auf die Anzahl sich bildender Neurone im Hippocampus von Labornagetieren aus. Laufradtests bei Labormäusen und Laborratten haben gezeigt, dass sich die adulte Neurogenese in dieser Hirnregion durch physische Aktivität mehr als verdoppeln lässt. Da Laufradaktivität auch mit verbesserter kognitiver Leistung und Lernfähigkeit der Versuchstiere assoziiert ist, liegt der Verdacht nahe, dass ein direkter Zusammenhang zwischen der Erhöhung der adulten Neurogenese und der verbesserten Hirnleistung besteht. Die Annahme dieses direkten Zusammenhangs führte zu grossen Hoffnungen im Kampf gegen neurodegenerative Erkrankungen. Somit könnte bei betroffenen Menschen, allein durch kontinuierliche körperliche Aktivität, die Anzahl neuer Neurone erhöht und dadurch dem kognitiven Abbau entgegengewirkt werden. Allerdings erschweren verschiedene Faktoren die simplistische Übertragung von Erkenntnissen aus Labortieren auf den Menschen. Die wenigen existierenden Studien in Wildtieren weisen auf starke artspezifische Unterschiede in der adulten Neurogenese, deren Regulierung und Funktion hin. Daher untersuchten wir, inwieweit unterschiedliche Umgebungen und Laufsituationen in Labormäusen und in nahe verwandten Wildmausarten die adulte Zellproliferation die und Bildung neuer Neurone im Hippocampus beeinflussen können. Die Untersuchung von verschiedenen Wildmäusen und Labortieren in der gleichen Umgebung erlaubt nicht nur eine artspezifische Analyse der adulten Neurogenese, sondern gibt auch Hinweise auf allfällige Domestikationseffekte in Labortieren.

Genetisch identische gleichaltrige Labormäuse des Stammes C57BL/6 zeigen individuelle Unterschiede in der adulten Neurogeneserate. Die prozentuale Reaktion der adulten Neurogenese auf freiwillige Laufradaktivität ist jedoch unabhängig von solch individuellen Unterschieden. Ändert man die Laufmotivation der Labormäuse dahingehend, dass das Laufen zu einer Art "Arbeit" wird, verliert sich der normalerweise beobachtete positive Effekt des Laufens auf die adulte Zellproliferation und Neuronenbildung. Auch konnten wir zeigen, dass eine gesteigerte Laufaktivität, durch eine Belohnung der Laufleistung, nicht zu einer zusätzlichen Erhöhung der adulten Neurogenese führt. Die Resultate lassen darauf schliessen, dass sich in Labormäusen der Kontext der externen Einflüsse entscheidend auf die Plastizität der adulten Neurogenese auswirkt.

Langschwanzwaldmäuse (*Apodemus sylvaticus*) und westeuropäische Hausmäuse (*Mus musculus domesticus*) sind mit den meisten Labormausstämmen genetisch nahe verwandt. Westeuropäische Hausmäuse gelten sogar als direkte Vorläuferspezies der Letzteren. Interessanterweise finden wir in beiden Wildspezies relativ stabile Anzahlen proliferierender Zellen und junger Neurone, die weder durch Laufradaktivität noch durch Stress oder eine stimulusarme Umgebung verändert werden können. Wildmäuse unterscheiden sich nicht nur in ihrer stabilen adulten Neurogenese von den Labortieren. Spezies-spezifische Unterschiede finden sich auch in der spontanen adulten Neurogeneserate.

Insgesamt zeigen unsere Versuche, dass die Plastizität der adulten Neurogenese im Hippocampus, sowohl in Labormäusen, als auch in Wildmäusen, eingeschränkt ist. In Labortieren finden wir zwar einen gewissen Bereich, in welchem die adulte Neurogenese durch Laufaktivität moduliert werden kann, dieser ist aber stark abhängig von der Laufmotivation der Tiere. In einer kontinuierlich wechselnden Umgebung wäre eine plastische Anzahl junger Neurone nicht von Vorteil, da eine ständige Anpassung der Zellzahl an die Umgebung mit hohen energetischen Kosten verbunden wäre. Wir vermuten deshalb, dass die adulte Neurogenese in Wildtieren genetisch auf einem konstanten Level gehalten wird. In domestizierten Labormäusen hingegen, scheint ein Verlust der homöotischen Kapazität in der Regulierung der adulten Neurogenese wahrscheinlich. Die artspezifischen Unterschiede nicht nur im Bereich der Modulation, sondern auch im Bezug auf die spontane adulte Neurogeneserate unterstreichen die Schwierigkeit, Erkenntnisse aus einer Spezies auf eine andere zu übertragen.

ABSTRACT

Evidence for the generation of young neurons out of precursor cells in the adult brain, i.e. adult neurogenesis, exists for at least two brain regions. New nerve cells are generated in the subventricular zone of the olfactory bulb and in the subgranular zone of hippocampal dentate gyrus. Young neurons of the subgranular zone migrate along the rostral migratory stream to the olfactory bulb, where they functionally integrate and contribute to the discrimination of odors. In the hippocampus the function of newly formed granule cells is still a matter of debate, yet it is thought that adult neurogenesis functionally contributes to hippocampal functions.

Over the last twenty years of extensive research it became clear that adult hippocampal neurogenesis (AHN) in laboratory rodents can be up-and down regulated by different internal and external stimuli. Physical exercise in a running wheel being among the factors that have been most investigated. Since voluntary exercise not only increases adult neurogenesis in the hippocampus but also beneficially affects learning and memory in laboratory mice and rats, a widespread assumption holds a direct relationship between AHN and cognitive brain health also in higher order species, including humans. However, translating findings in laboratory rodents to the human condition faces difficulties. Enormous differences in basal rates of adult neurogenesis have been reported between mammalian species. The low level of AHN in primates and the complete lack of adult neurogenesis in bat species indicate species-specific differences in adult neurogenesis not only on a regulatory but also on a functional level. For a better understanding of species-specific differences in the regulation of AHN, we investigated basal rates of adult neurogenesis in laboratory mice and closely related wild mouse strains as well as the reaction of AHN to motivationally different running conditions. Testing different wild- and laboratory mice in the same environment allowed the identification of species-specific differences as well as possible domestication effects.

Basal rates of adult hippocampal neurogenesis in equally-aged and genetically identical laboratory C57BL/6 mice show individual differences possibly reflecting epigenetic factors. However, the initial level of adult neurogenesis does not influence the response to wheel-exercise. Voluntary physical exercise in laboratory mice always increases AHN but this positive effect cannot be additively stimulated by enhanced running and is even lost as soon as the mice are forced to run. Rewarding the mice for their performance leads to an increase in wheel activity

but does not translate into a corresponding additive increase in adult neurogenesis. Likewise, a more naturalistic situation, in which laboratory mice must run to obtain their daily food does not lead to an increase in cell proliferation and entails only a small increase in the number of young neurons, far below the one in voluntary running mice.

Wild wood mice (*Apodemus sylvaticus*) and wild-derived western house mice (*Mus musculus domesticus*), both close relatives of the common laboratory mouse strains, were tested in the same running situations as laboratory C57BL/6 mice. Besides species-differences in basal neurogenesis rate, we find adult neurogenesis in wild mice remaining relatively constant in response to external influences. None of the factors that normally affect AHN in laboratory animals, such as stress, environmental changes or physical exercise, have an effect on adult neurogenesis in these animals. In wood mice, neither voluntary wheel running nor stress or an impoverished cage environment affect the number of newly generated neurons. House mice also show a stable adult neurogenesis, which shows no significant change after voluntary running or running for food.

Adult neurogenesis in the dentate gyrus is thus regulated differently in laboratory and wild mice. However, even in laboratory mice it is not as plastic as initially suggested: laboratory mice, which are tested in a more naturalistic and complex running situation, show rather weak plasticity of AHN, resembling wild mice. Hence, it seems that the regulatory difference in adult neurogenesis between laboratory- and wild mice is, that laboratory animals react to a single stimulus in absence of other inputs. We believe that the constant exposure to different stimuli potentially affecting AHN has led to a natural selection that stabilizes adult neurogenesis in the wild. In contrast, during domestication - including inbreeding - much of the homeostatic capacity in regulating adult neurogenesis might have been lost.

Taken together, our data imply that genetic (species-specific differences as well as within-species variation) play an important role in determining basal rates of adult neurogenesis, while motivational-contextual factors modulate the response of AHN to physical exercise, albeit chiefly in domesticated laboratory strains . As such differences appear already between phylogenetically closely related species, extrapolating findings in laboratory mice to distantly related taxonomic groups, such as humans, obviously requires much caution.

ABBREVIATIONS

AHN: Adult hippocampal neurogenesis

BrdU: 5-bromo-2'deoxyuridine

GABA: γ-Aminobutyric acid

CA1, CA3: Regions of the Ammon's horn

EC: Entorhinal cortex

Sub: Subiculum

DG: Dentate gyrus

HVC: Higher vocal center

BDNF: Brain-derived neurotrophic factor

VEGF: Vascular endothelial growth factor

IGF-1: Insulin-like growth factor

BXD 2, BXD 8: Recombinant inbred lines of laboratory mice

C57BL/6, C57BR/CDJ, DBA, 129/SvJ, 129S1/SvImJ: Inbred laboratory mouse strains

SPF: Specific pathogen free

DCX: Doublecortin

N.A.: Numerical aperture

INTRODUCTION

1. Aims of the thesis

Almost half a century is past since the first descriptions of adult neurogenesis in the dentate gyrus of the hippocampus (Altman, 1962, 1963, Altman and Das, 1965) challenging the long believed dogma that neurons of the nervous system have no potential to regenerate. Although confirmed by several studies (Kaplan and Hinds, 1977, Kaplan and Bell, 1984), the presence of new neurons in this region did not attract scientific attention until the 1990s when a new staining method with the thymidine analogue BrdU allowed the detection and tracking of this special population of new cells (Kuhn et al., 1996). During the last twenty years adult hippocampal neurogenesis (AHN) has been described in a large number of mammalian species, including new world (Gould et al., 1998, Bunk et al., 2011)- and old world primates (Gould et al., 1999b, Jabes et al., 2010) as well as humans (Eriksson et al., 1998). Much attraction has gained the finding that in laboratory rodents adult neurogenesis can be modulated by internal and external factors, such as growth factors (Cotman et al., 2007), stress (Gould and Tanapat, 1999), environmental enrichment (Kempermann et al., 1997b) and physical exercise (van Praag et al., 1999b). Physical exercise is one of the most investigated modulators of AHN as it has the potential to massively increase the number of young neurons. Because running is also beneficial for spatial learning and cognitive abilities (van Praag et al., 1999a, van Praag et al., 2005), a direct link between adult hippocampal neurogenesis and learning and memory seems likely. This association has raised hopes in the treatment of neurodegenerative disorders in humans but a simplistic translation from findings gained almost exclusively from laboratory animals to the human condition is questionable. The few studies about adult neurogenesis in wild living mammals and rodents show that adult neurogenesis levels differ considerably between species (Amrein et al., 2004b, Barker et al., 2005, Amrein et al., 2007, Klaus and Amrein, 2011). Some species that are naturally very active show low or even no AHN challenging the conclusions derived from exercise related findings in laboratory animals (Amrein et al., 2007, Bartkowska et al., 2008). These species-specific differences in adult neurogenesis suggest that genetic factors play an important role also in response of AHN to physical exercise.

The aim of the present work is to gain insight into the differences in modulation of adult neurogenesis between genetically closely related laboratory and wild mouse strains while testing

them under the same experimental running conditions. We not only wanted to test whether AHN of wild mice can be modulated by physical exercise, but also how adult neurogenesis of laboratory mice reacts to running conditions mimicking a more naturalistic situation.

2. The hippocampus

The hippocampus is one of the evolutionary oldest cortical brain structures located along the lateral cerebral ventricles (Fig. 1). Belonging to the limbic system, the hippocampus is a structure functionally associated with memory formation and long-term memory consolidation.

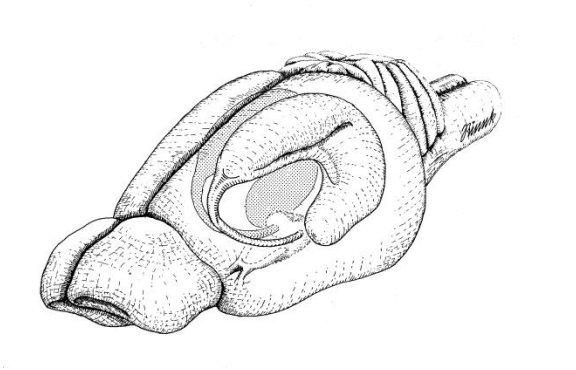

Fig. 1

Schematic drawing of a rat hippocampus following the lateral cerebral ventricles.

2.1. Anatomy

Information processing in the hippocampus happens largely unidirectional via a trisynaptic excitatory pathway (Andersen et al., 2006) (Fig. 2). The port of entry for processing cortical information is the molecular layer of the dentate gyrus where information arrives topographically structured via the perforant path originating from the entorhinal cortex. The dentate gyrus mainly consists of excitatory glutamatergic granule cells extending their apical dendrites into the molecular layer where they get input from two distinct projections from the lateral and medial entorhinal cortex. The dentate granule cells send thinly myelinated axons, called mossy fibers, into Ammon's horn where they form glutamatergic connections with pyramidal cells of the region CA3 and synapse with GABAergic interneurons. The dendrites of the CA3 pyramidal cells arborize above and below the cell bodies where they connect with two distinct mossy fiber projections. The terminal field of the mossy fibers contacting with the apical dendrites is called the suprapyramidal projection and the terminal field of mossy fibers contacting with the basal dendrites is called the infrapyramidal projection. CA3 pyramidal cells send axon projections called Schaffer collaterals into region CA1, where they synapse preferentially with the apical dendrites of CA1 pyramidal cells. The pyramidal cells of CA1 project back to the subiculum which then, in turn, projects topographically structured information back to the entorhinal cortex and to different brain stem nuclei (Namura et al., 1994) finishing the trisynaptic loop. The

entorhinal cortex is strongly and reciprocally connected with many other parts of the cerebral cortex, chiefly higher-order association cortices.

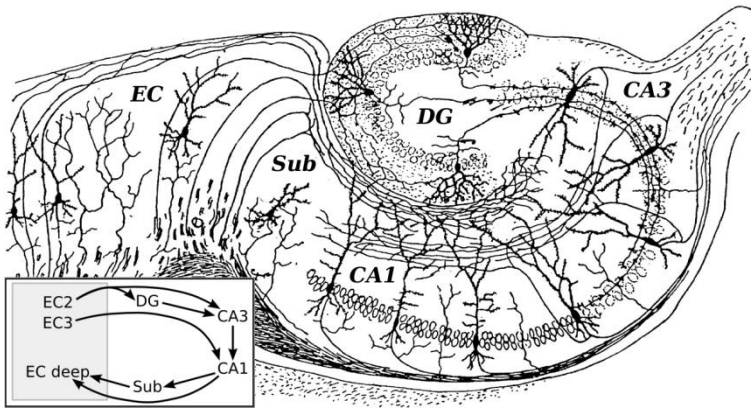

Fig. 2

Schematic drawing of the trisynaptic information processing in the mammalian hippocampus by Santiago Ramon y Cajal (Ramón y Cajal, 1911). EC: Entorhinal cortex; Sub: Subiculum; DG: Dentate gyrus; CA1, CA3: Regions of the Ammon's horn

Of note is that this description of the unidirectional hippocampal information transmission is simplified. The entorhinal cortex, for example, not only synapses to the dentate gyrus. Specific topographically arranged projections from layer III of the entorhinal cortex synapse to the apical dendrites of pyramidal cells of CA1 and to the subiculum. This monosynaptic information flow is however of minor importance for the present work.

2.2. Functional aspects of the hippocampus

The primary function of the hippocampus is the organization of memories. Main insights into the involvements of the hippocampus in memory function have been gained from famous patient H.M. whose hippocampus was removed in attempt to cure epileptic seizures (reviewed in (Corkin, 2002)). Unexpectedly, the surgical outcome revealed a severe anterograde amnesia pertaining to the long-term declarative memory, meaning that the patient was unable to form new episodic or factual memories and to remember events that happened just before his surgery. However, the patient retained declarative memories for things that happened earlier and he had also an intact non-declarative memory, which means that he was able to learn and memorize new skills (Gabrieli et al., 1988). Other case studies confirmed the findings of patient H.M. and strong evidence has accumulated that the hippocampus plays a key role in declarative memory function, i.e. the formation of memories that can be consciously recalled (Scoville and Milner, 2000). Further it became clear that the role of the hippocampus in memory function has a time limitation. Squire and Alvarez created the idea of a systems-level memory consolidation process.

Once initial memory has formed in the hippocampus a gradual reorganization (consolidation) of this memory takes place over the period of learning. After this consolidation process long-term memory tracers are stored in the cortex and the hippocampus is no more required for recalling a particular memory (Squire and Alvarez, 1995).

Another important functional role that has been attributed to the hippocampus is spatial learning and navigation in rodents. A specific spatial system in animals is thought to be required for the formation of new declarative memories. Studies in freely moving rodents have shown that granule cells of the hippocampal dentate gyrus and pyramidal cells of the hippocampal CA3 and CA1 region fire according to the animal's location in the environment. These so called "place cells" fire bursts of action potentials while an animal passes through a particular part in the spatial environment. The "place field" of a single place cell is the region in which the cell fires the most (O'Keefe and Dostrovsky, 1971, O'Keefe, 1976). The firing rate of a place cell increases from less than one spike every 10 seconds (1Hz) outside its corresponding place field to a maximum rate of 40Hz in the center of its place field. Every single place cell is attributed to a specific location in the environment, in which it fires the most. Together these cells are able to build a neural layout of any given environment. The size and also the shape of place fields varies with size and shape of the actual environment and gradually along the length of the hippocampus (Maurer et al., 2005). The differences in place field sizes are of importance in view of the functional differences between the ventral and dorsal part of the hippocampus. Cells with place fields that cover almost the entire testing environment might have a more contextual function, i.e. distinguishing between two different testing environments. In contrast cells with small place fields enable identifying more specifically different locations within a testing area. Evidence from place cells strongly supports the involvement of the hippocampus in spatial mapping and memory formation in rodents.

3. Adult Neurogenesis in the hippocampal dentate gyrus

Adult neurogenesis is the process of neuronal proliferation, differentiation and integration into the existing neuronal network after the fetal and early postnatal development. This phenomenon has been described in different brain regions of many vertebrate species (reviewed in (Ming and Song, 2005)). In lower vertebrates like fishes and reptiles a continuous production of neurons leads to a lifelong growth of various brain areas (Garcia-Verdugo et al., 2002). Adult neurogenesis in birds has also been observed in different brain regions without alterations of structural volumes over time, including the higher vocal center (HVC) of the birdsong system (Kirn and Nottebohm, 1993) and the avian hippocampus (Barnea and Nottebohm, 1996). Interestingly, both of these regions are involved in different kinds of learning procedures, indicating a functional contribution of the newly generated neurons in these processes. Adult neurogenesis in the HVC for example is subject to seasonal fluctuations with higher amounts of young neurons during periods of higher levels of song learning (Kirn et al., 1994, Wilbrecht et al., 2006).

In mammals adult neurogenesis is limited to the subventricular zone of the olfactory system and the subgranular zone of the hippocampal dentate gyrus, although few studies report the presence of young neurons in distinct cortical regions (Gould et al., 1999c, Ponti et al., 2008). Neurons generated in the subventricular zone of the olfactory system migrate along the rostral migratory stream to the olfactory bulb where they integrate into the persistent neuronal network and become granular or perigranular interneurons expressing a variety of chemical phenotypes (Bedard and Parent, 2004, Winpenny and Raineteau, 2010). Functionally these interneurons may contribute to chemorezeption, as a diminished number of young cells in this region leads to impaired odor discrimination (Gheusi et al., 2000). In the hippocampus the functional contribution of young neurons during adulthood remains an issue of debate. There is no doubt that also in this region young neurons become functionally active, as studies were able to electrophysiologically prove their functionality after integration into the network (Jessberger and Kempermann, 2003, Bruel-Jungerman et al., 2007). Yet it is still unclear whether young neurons in the hippocampus functionally act as a distinct population or are contributing to the functionality of the whole hippocampal system (Bruel-Jungerman et al., 2007). Intuitively, it has been suggested that new neurons contribute to hippocampal memory function but findings are contradictory (Leuner et al., 2006). Studies that report positive correlations between the number of newly formed neurons and

memory performance in learning tasks (van Praag et al., 1999a, Drapeau et al., 2003, Dupret et al., 2008, Thuret et al., 2009) imply a direct relationship between AHN and memory, although this relation is not necessarily causal. In contrast reports which are not able to find an association between spatial learning and adult neurogenesis (Meshi et al., 2006, Jaholkowski et al., 2009) question this generalized and intuitive view. Jaholkowski and colleagues summarized the controversial findings in mice and rats after deletion of AHN through irradiation or genetic ablation (Jaholkowski et al., 2009). More than half of the studies they cited do not report an association between adult neurogenesis and learning. This summary, however, needs to be taken carefully because of confounding factors like species, learning task and ablation model. The extent to which adult neurogenesis is mediating learning has been shown to differ between mice and rats (Snyder et al., 2009a) and also AHN has been shown to be associated with only specific forms of learning (Kempermann and Gage, 2002a). Not every form of hippocampus dependent learning can be correlated with adult neurogenesis, which seems quite reliable in view of the complexity of the hippocampal structure. During the last two years evidence has accumulated that AHN functionally contributes to hippocampal spatial pattern separation, which is the ability to discriminate between stimuli with high temporal and spatial similarity (Clelland et al., 2009). After more than 20 years of extensive research there is still no clear answer why new neurons are generated in the hippocampal dentate gyrus and what their functional benefit exactly is.

The following paragraphs will focus on adult hippocampal neurogenesis (AHN) only. The term adult neurogenesis will therefore only be used in relation to the hippocampus.

4. Exercise as a modulator of adult hippocampal neurogenesis in laboratory rodents

An interesting finding about AHN is that in laboratory rodents the amount of cells that divide and differentiate into young neurons can be modulated by internal and external stimuli. Internal levels of growth factors like BDNF, VEGF of IGF-1 influence adult neurogenesis (Cotman et al., 2007) as well as circulating hormones like estrogen (Tanapat et al., 1999, Ormerod et al., 2004). Additionally age and external factors such as stress, environmental enrichment or wheel-running can dramatically change the number of newly generated neurons. Whereas age (Ben Abdallah et al., 2010) and stress (Gould and Tanapat, 1999, Mirescu and Gould, 2006) have been shown to decrease adult neurogenesis, environmental enrichment (Kempermann et al., 1997b) and the use of running wheels have the opposite effect (van Praag et al., 1999b).

Voluntary physical exercise is one of the most extensively investigated stimulators of AHN. In laboratory mice and rats it has the potential to increase the level of dividing and neuronally differentiating cells up to three-fold, yet remarkable strain differences in the running-induced response of adult neurogenesis have been detected (see chapter 4.2.). The increase, however, seems to be transient, tapering off after three weeks of running (Naylor et al., 2005, Snyder et al., 2009b, Clark et al., 2010). Unlike environmental enrichment, which induces enhanced AHN by increasing the survival rate of the newly generated neurons, physical exercise mainly increases the number of proliferating cells and thereby also the number of young neurons being formed. The two forms of increased adult neurogenesis have been explicitly separated (Olson et al., 2006), however, recent studies as well as our own findings show that voluntary exercise can have an effect on the survival of immature neurons as well (Klaus et al., 2009, Snyder et al., 2009b, Klaus et al., 2011). Most likely the increase of cell proliferation and adult neurogenesis through voluntary running is mediated by enhanced levels of growth factors like IGF-1 or VEGF. Exercise induces increased levels of IGF-1 and VEGF in the periphery. Both growth factors are able to reach the brain by crossing the blood-brain-barrier. Blocking either IGF-1- or VEGF-entry into the brain inhibits the running-induced increase of proliferation in the hippocampus (reviewed in (Cotman et al., 2007)).

4.1. The relation between adult neurogenesis, exercise and cognition

Besides the positive influence that physical exercise has on the number of proliferating and neuronally differentiating cells in the hippocampus, it can also positively affect cognitive abilities

of laboratory rodents in spatial and non-spatial learning tasks (Samorajski et al., 1985, Fordyce and Wehner, 1993, Adlard et al., 2004, Eisenstein and Holmes, 2007, Garcia-Capdevila et al., 2009). Many researchers have intuitively concluded that the improved cognitive performance might be the consequence of the running-increased numbers of proliferating and neuronally differentiating cells, yet the rare studies investigating the direct relation between all three factors comprise contradictory results. Correlations between running-increased AHN and cognitive improvements have been found in the Morris water maze task in adult- as well as aged C57BL/6 mice (van Praag et al., 1999a, van Praag et al., 2005) and, more recently, in pattern separation tasks (Creer et al., 2006). Contrasting results have been found, for example, by Wojtowicz and colleagues reporting no significant effects of running-increased adult neurogenesis on behavioral performance in the Morris water maze task and in the contextual fear conditioning task in rats (Wojtowicz et al., 2008). Another interesting study reports the interactions between exercise, adult neurogenesis and cognitive improvements in the water maze to depend on the genetic background of the mice (Llorens-Martín et al., 2010). The dependency of a possible correlation on the genetic background is also underlined by a work of Rhodes and colleagues who showed that the correlation between running-increased AHN and performance in the water maze is lost in mice selected for high wheel performance (Rhodes et al., 2003). Summarizing the results of these studies, it gets apparent that the relation between exercise, adult neurogenesis and cognition might be rather complex, depending on different external and internal conditions (Fig. 3). The genetic or epigenetic setup of the species or the laboratory strain might play an important role in determining the correlational relationship between exercise, AHN and cognition as well as the context of the learning task. Potential contextual and genetic effects will be discussed in more detail below.

An issue which also needs to be considered is, that both, exercise-increased adult neurogenesis as well as exercise-induced improvement in cognitive performance, have been shown to correlate with exercise-enhanced levels of growth factors like BDNF, VEGF or IGF-1 (Vaynman et al., 2004, Gobbo and O'Mara, 2005, Trejo et al., 2008). In view of this background, the intuitive conclusion that the running-induced improvement in performance causally derives from an increase in AHN could therefore be a misinterpretation. Exercise might rather be considered as a trigger which, again depending on factors like strain or context, induces transcriptional cascades that have the potential to simultaneously increase adult neurogenesis and cognition (Fig. 3).

Physical activity may act in a much broader sense on molecular, cellular and system level (reviewed in (Lista and Sorrentino, 2010)) both on a phylogenetic and ontogenetic scale.

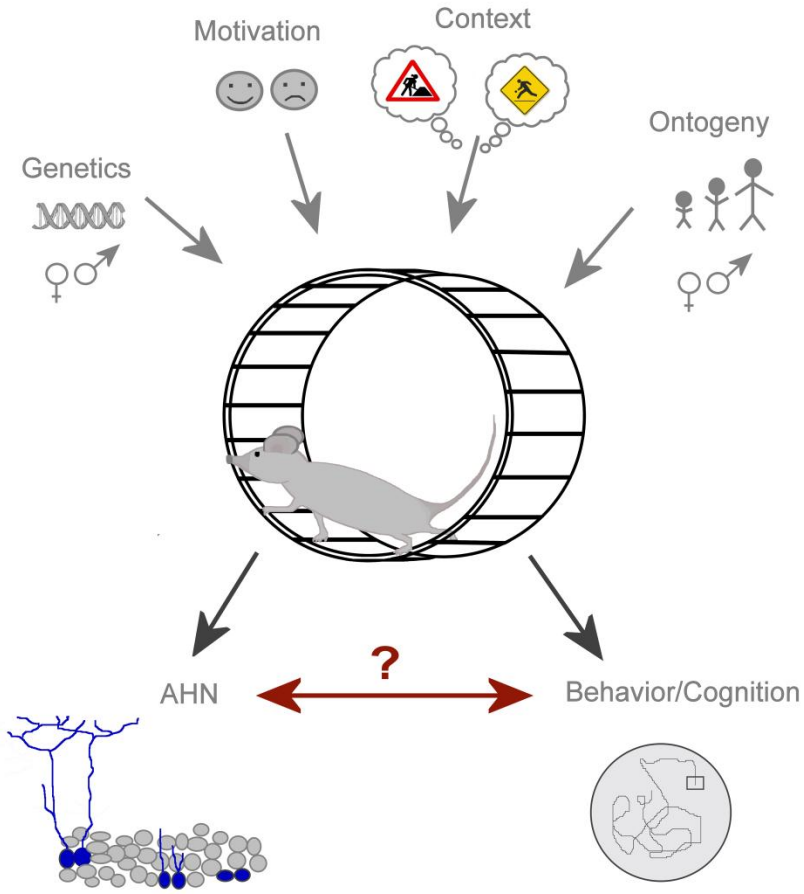

Fig. 3

Overview over possible external and internal factors that potentially influence cognition and the level of neurogenesis in running laboratory rodents. These external factors may determine the correlation between running, neurogenesis and cognition.

4.2. Genetic variability of exercise and adult neurogenesis

As already mentioned above, the extent to which AHN can be up-regulated by exercise, depends, amongst others, on the genetic background of the animals. The reason why the modulation of adult neurogenesis differs across species, may be, that basal rates of AHN themselves, including cell proliferation, neuronal differentiation and survival, are regulated strictly species-specifically. Different laboratory mouse and rat strains, for example, differ remarkably in basal rates of adult neurogenesis (Kempermann et al., 1997a, Perfilieva et al., 2001, Kempermann and Gage, 2002b) and recombinant inbred strains of DBA and C57BL/6 mice show large differences in cell proliferation, neuronal differentiation, survival and in their relations (Kempermann and Gage,

2002a). This latter study highlights the underlying genetic potential that is able to determine neurogenic processes in a variety of ways and which likely also includes the possibility to react diversely to external stimuli.

The degree of voluntary wheel running has a genetic component as well (Lightfoot et al., 2010). Large variations in spontaneous voluntary running performance can be found in strains of laboratory mice (Festing, 1977, Lerman et al., 2002) and rats (Johnson and Mitchell, 2003). Female mice have been reported to generally run longer distances than males, yet the difference reaches statistical significance only in a few strains but not including the commonly used laboratory strains as C57BL/6, DBA or 129/SvJ (Klaus and Amrein, 2011). Notably, different research groups have reported different levels of voluntary running for the same laboratory strain. For example, female C57BL/6 mice investigated in our laboratory run ~10km/day (Klaus et al. 2011), whereas other research groups found higher (De Bono et al., 2006) or lower (Lightfoot et al., 2004) performance in similarly-aged mice. These differences might be related to differences in housing conditions (see Unpublished findings) and/or differences in the strength of the running-wheel as we found the genetic background to determine running performance rather tightly. We tested equally aged C57BL/6 mice of different suppliers in the same running environment using the same running wheels. In contrast to adult neurogenesis (see Unpublished findings) we did not find supplier-related differences in the overall running activity and running performance.

Interestingly, species with similar running performances can vary considerably in their basal adult neurogenesis rate implying that species-specific performance under voluntary conditions does not correlate with species-specific levels of AHN. For example C57BL/6 mice perform at a similar level than 129/SvJ mice but show a much higher basal AHN (Kempermann et al., 1997a, Kempermann and Gage, 2002b, Lightfoot et al., 2010). Of note is the observation that the range of variation among mouse strains appears similar both for basal adult neurogenesis rate and for voluntary wheel-running performance. The ~27-fold difference in daily running performance between the lowest running mouse strain (129S1/SvImJ) and the highest scoring strain (C57BR/CDJ) (Lightfoot et al., 2010) is comparable to the 26-fold difference in adult neurogenesis between 13 recombinant inbred substrains derived from C57BL/6 and DBA/2 mice, among which BXD-2 and BXD-8 hold the extreme positions (Kempermann and Gage, 2002a).

On the other hand, the variation in both traits appears poorly correlated, arguing again against a simple mechanistic quantitative relation between voluntary wheel-running and adult neurogenesis

4.3. Context-dependency of running-induced adult neurogenesis

The positive effect of running can vary depending on the context of the running conditions, often with respect to motivation and mood. Social isolation of rats for example is sufficient to prevent the positive effect of exercise on AHN (Stranahan et al., 2006). In addition, conditions which create a necessity for the animals to run or motivate the animals to run above their voluntary level do not lead to an enhanced adult neurogenesis (Kim et al., 2003, Klaus et al., 2009, Klaus et al., 2011). Forced treadmill running in rats loses its beneficial effect on the number of young neurons if the treadmill speed exceeds a moderate level (Kim et al., 2003). Related to this finding treadmill running has been found to have no or even negative effects on cognitive learning abilities (Blustein et al., 2006). The contextual difference between voluntary exercise and the inescapable forced treadmill condition gets even clearer when comparing different mouse strains in their ability to perform in these running tasks. C57BL/6 mice, for example, perform on a very high level under the voluntary running condition but do worse in the treadmill (Lerman et al., 2002). More evidence exists that, in mouse and rat strains, speed and distance in voluntary running do not correlate with endurance and maximally tolerated speed in the forced treadmill task (Lambert et al., 1996, Lerman et al., 2002). Given these findings, we designed two running paradigms with different contextual aspects. Chocolate-rewarding the mice for their performance led to a significant increase in the level of running but this increase did not entail rates of AHN higher than in voluntary running mice (Klaus et al., 2009). Likewise, a rather naturalistic running paradigm, in which the mice had to run for their daily need of food, did not lead to changes in running activity compared to voluntary running mice, but the stimulating effect on cell proliferation, that running normally induces, was lost under these conditions and the number of young neurons was significantly lower than in voluntary running animals (Klaus et al. 2011).

Kim and colleagues suggest that the loss of the effect of treadmill exercise on AHN with enhanced speed is due to an increased stress level of the animals (Kim et al., 2003). In general, stress and the concomitant elevation in circulating stress hormones have been associated with a decrease in adult neurogenesis (Gould and Tanapat, 1999, Mirescu and Gould, 2006). Interestingly, voluntary running itself elevates circulating glucocorticoid hormones but

nevertheless enhances the number of young neurons in the dentate gyrus (Stranahan et al., 2008). We believe that the emotional valence of the context may act as a switch to decide whether enhanced glucocorticoid levels negatively or beneficially influence adult neurogenesis. This hypothesis is further supported by a non-exercise study, in which enhanced glucocorticoid levels through mating behavior also positively influence AHN (Leuner et al., 2010).

Summarizing the effects of the different running paradigms on adult neurogenesis, it gets apparent that the link between running and AHN is not as simple as initially presumed. The emotional valence of a given context should be carefully considered.

4.4. Species-specific differences in the regulation of adult neurogenesis - Running wild

A running-associated increase in AHN has been observed in most laboratory mouse- and rat strains, albeit not to equal extents. This contrasts with the findings in wild- or wild-derived mice, which do not show an increase in cell proliferation and neuronal differentiation after running wheel exercise. Wild wood mice (Fig. 4), which are close relatives of the common laboratory inbred strains, show a very stable adult neurogenesis that does neither react to wheel-exercise nor to impoverished environment (Hauser et al., 2009). Additionally, the F1 generation of wild-derived house mice (Fig. 4), which are the closest relatives of the laboratory mice sharing 92% of their genome with C57BL/6 mice (Yang et al., 2007), does not show a significant effect on adult neurogenesis after two weeks of voluntary wheel-running (Klaus et al. 2011). Likewise, in other wild-living species AHN appears to be resilient to modulatory effects. Squirrels, for example, show stable adult neurogenesis in spite of seasonally changing requirements in spatial memory processing (Lavenex et al., 2000). The only factor that has been shown to influence the number of proliferating and neuronally differentiating cells in the hippocampus of wild rodents is age, although even the age-related decline in AHN has been shown to depend on the species (Amrein et al., 2004b). Thus, aside from age, wild and wild-derived rodents show adult neurogenesis levels that do not respond, or respond only weakly, to changes in activity, environmental complexity, context or cognitive requirements.

Laboratory animals are isolated from environmental stimuli which, under natural conditions, promote survival. The natural environmental complexity, in which foraging, predator risk, intra- and interspecies interactions play an important role, is largely absent under laboratory conditions. Interestingly, many of the factors, that wild mice under natural conditions are constantly exposed

to, are known to potentially modulate adult neurogenesis in laboratory rodents. Environmental and social changes (Kempermann et al., 1997b, Leasure and Decker, 2009), hormonal fluctuations (Tanapat et al., 1999), extended locomotor activity (van Praag et al., 1999b) and aggressive behaviors (Fiore et al., 2005) are only a few factors that have been shown to modulate AHN in laboratory animals. Given that adult neurogenesis is thought to functionally contribute to cognitive abilities, one might intuitively conclude that rodents experiencing a naturally complex environment would show higher AHN than their laboratory relatives, leading to a saturated level of adult neurogenesis which cannot be further modulated by stimulating factors like wheel-running. Laboratory rodents would therefore display levels of adult neurogenesis underestimating the normal (natural) level of cell proliferation and neuronal differentiation. However, contrasting this intuitive assumption, wild mice (Amrein et al., 2004b, Klaus et al., 2011), rats (Epp et al., 2009), voles (Ormerod et al., 2004), squirrels (Lavenex et al., 2000) and chipmunks (Barker et al., 2005) can have lower, similar or higher baseline levels of adult neurogenesis than laboratory rodents. Stable AHN in wild species can therefore not be related to a species-specific ceiling level of adult neurogenesis. The picture gets even more complex when including wild species that do not show AHN during all (Microchiroptera (Amrein et al., 2007)) or only during parts of their life (Shrews (Bartkowska et al., 2008)).

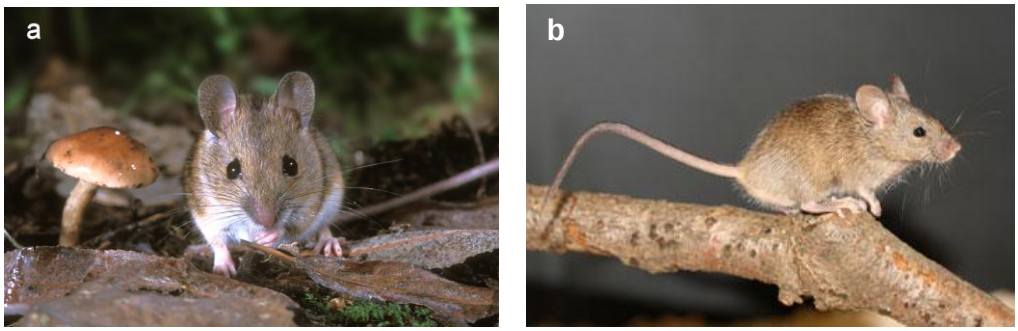

Fig. 4

a: Long-tailed wood mouse (*Apodemus sylvaticus*) (Rollin Verlinde, with permission from vilda (www.vildaphoto.net))
b: Western house mouse (*Mus musculus domesticus*) (MPI für Evolutionsbiologie, http://idw-online.de/pages/de/news334630).

Apparently, adult neurogenesis in wild living animals is regulated differently from laboratory animals. In wild-living rodents the maintenance of a relatively constant pool of proliferating and

differentiating neurons could be a strategy for long-term stable regulation of AHN in a constantly changing environment. Alternatively, loss of genetic heterogeneity during domestication, or specific domestication-related at the genetic level, might have impaired the efficiency of homeostatic regulation loops. Whatever explanation applies, most important in the context of species-specific regulation of adult neurogenesis is, that the genetic variation between and within taxonomic groups differs over at least one order of magnitude, which by far exceeds the effect of any experimentally induced change, including voluntary exercise.

UNPUBLISHED FINDINGS

1. Effect of different environments on wheel activity

1.1. Introduction

Laboratory mice and rats have been shown to spontaneously and extensively start to run when provided with a running wheel. Most research groups report a strong increase in running performance during the first two weeks of voluntary exercise, which then reaches a plateau (Ferreira et al., 2006, Brene et al., 2007). This drastic increase in performance during the first week has been related to an addictive response as the dopaminergic reward system is activated similarly to drug consumption (Brene et al., 2007). We show that only animals subject to a standard environment, i.e. to a laboratory facility in which different mouse strains are kept together, show the expected initial increase in performance. In contrast, mice that were habituated and tested in a quiet environment, without disturbing external influences, started on a higher running level and displayed a lesser increase of performance. Our results indicate that the increase in performance and therewith also the possible addictive response during the initial running period strongly depends on the experimental environment. The latter may have a strong influence on the emotional state of the animal and concomitantly also on the experimental outcome.

1.2. Methods

Prior to running, 12-week old female C57BL/6 mice (Labortierkunde, Universität Zürich) were habituated in groups of six to an inverted light dark-cycle and to their novel experimental environment. One half (6 mice) of the animals was habituated in a separate room without disturbing influences from other animals or laboratory personal (quiet environment group). The other half (6 mice) was acclimatized in a common room of the standard non-SPF animal facility together with mice of different species and mixed genders (standard environment group). After one week of acclimatization the mice were individually subject to running-wheel-equipped standard laboratory cages. The experimental environment for both running groups remained the same as during habituation. Running wheels of each cage were connected to a controller system (AMS Software & Electronic GmbH, Flensburg, Germany) which enabled us to record the

activity of the mice in one-hour bins. Recording of wheel-revolutions started after one day of acclimatization to the new cage-environment. Data were collected once a day in the middle of the dark phase.

Statistical analyses were performed with the SPSS 19.0 statistical Software (SPSS Inc, Chicago, Illinois). One-way ANOVA was used to assess group differences in running levels on a daily as well as on a weekly basis.

1.3. Results

Animals which were habituated and tested in a quiet environment showed a daily activity pattern that is related to the animal's natural sleep and wake rhythm. In contrast, we found the daily wheel activity pattern of the standard environment group to be very inconsistent with no circadian-related distribution (Fig. 5a). The only inconsistency in the quiet environment group was found around 13:00 h when a massive drop in wheel activity was recorded in all animals of the corresponding group. This inconsistency can be related to the fact that, during this time, animals were disturbed for data recording. The inconsistent daily activity pattern of the standard environment group disappeared over the experimental period ending in a comparable activity pattern to that of quiet environment runners (Fig. 5b).

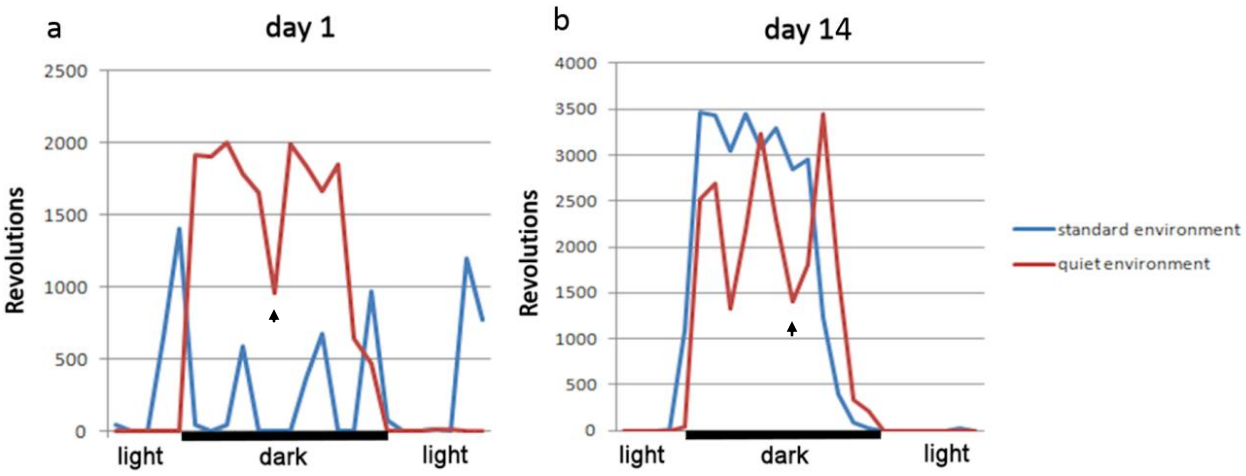

Fig. 5

Hourly running-activity on the first- (a: **day 1**) and last (b: **day14**) day of recording of two animals either tested in a quiet- or a standard environment. The time of reading controllers is indicated by an arrow. In both figures the same animals are depicted.

Animals in the standard environment started on a significantly lower running level than mice in the quiet environment (p=0.001) but they rapidly increased their daily performance (Fig. 6). After reaching the performance level of the quiet runners on recording day five, standard environment animals exceeded the performance of the quiet environment runners on every consecutive day (Fig. 6). The average performance of the standard environment runners is significantly higher than that of quiet environment animals (p=0.044). On average both running groups run significantly more during the second week of running (quiet environment: p=0.015, standard environment: increase p=0.002). The increase is, however, less in the quiet environment runners (36%) than in the standard environment runners (110%).

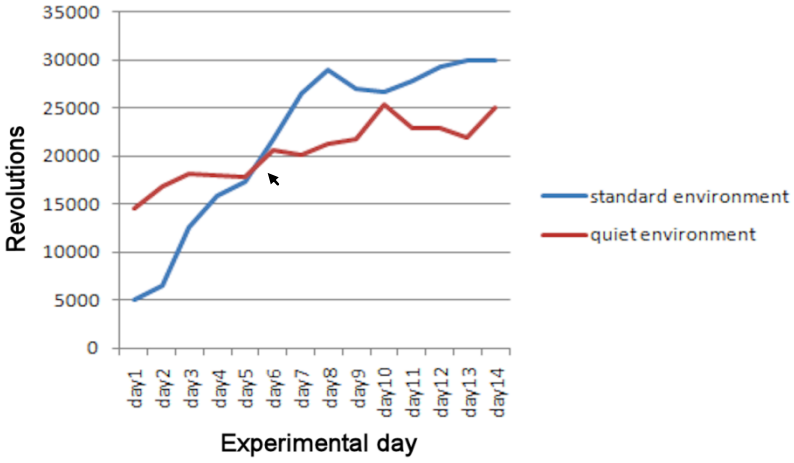

Fig. 6

Average daily running-activity of a group of animals being either habituated to a quiet environment or a disturbed environment together with mice of different strains and gender (standard environment). On recording day five the groups overlapped in their performance level (indicated by an arrow).

1.4. Conclusion

We find running patterns of the two running groups to differ significantly on an hourly as well as on a weekly basis. Whereas animals, habituated and tested in a quiet environment, show an activity pattern that is related to the animal's sleep-wake cycle, animals, which were habituated and tested in a disturbed environment, show peaks of running activity distributed over 24 hours. The inconsistent activity pattern in standard environment mice is stabilized during the running experiment, adapting more and more to an appropriate sleep-wake rhythm. However, the overall performance curve of the standard habituation group displays a significantly steeper increase in activity during the first week of voluntary exercise compared to animals that were subject to a quiet environment.

We conclude that disturbing factors like odors and sounds of different kinds of mice as well as the odor and high-frequency noise of pups has disrupted the normally observed nocturnal activity pattern under wheel-running conditions of our standard habituation mice. Nocturnal wheel running is commonly used as readout of the circadian system in chronobiological research. The presence of males and pups in the same environment might have imposed a stress to our female subjects, which did affect the animals sleep-wake cycle. Different kinds of stressors have been shown to impair circadian-related mechanisms in the brain, leading for example to impairments in light-induced circadian clock-resetting (Amir and Stewart, 1998). Additionally, Solberg and colleagues reported an altered percent distribution of total activity and an increased fragmentation of the daily activity rhythms during a period, in which the mice were subject to chronic stress (Solberg et al., 1999). Thus, it is likely that in our mixed habituation mice physiological processes mediating circadian rhythmicity were impaired. The fact that during two weeks of voluntary exercise the sleep-wake disruption was reversed could be related to a stress-preventing effect of physical exercise itself, which has been reported in many studies (Solberg et al., 1999, Greenwood et al., 2003).

Curiously, the expected increase in running during the first week of voluntary exercise, which has been reported in many studies, is almost missing in our quiet habituation group but very prominent in the standard habituation mice. Studies which report this pronounced increase in performance during the initial phase of voluntary running, often relate this phenomenon to an addiction effect (Brene et al., 2007). This is also supported by the finding that the mesolimbic dopaminergic system during exercise reacts similarly than during drug intake (Werme et al., 2002). Data of the present study question this theory by showing only a very mild increase in running in the quiet habituation mice. We suppose that, in the latter case, the increase in performance might rather be related to motor learning than to addictive behavior. The very drastic increase in running activity in the mixed habituation group, however, could indeed be related to addictive behavior as stressed animals are much more prone to stereotypic behavior by altering the sensitivity of dopaminergic receptors (Cabib et al., 1984).

In summary, different habituation environments are able to powerfully impact on the animal's activity pattern and thereby significantly influence the outcome of an experiment. We therefore think that circumstances of habituation and experimental environments should be carefully

mentioned in every materials and methods section in order to being able to compare different studies.

2. Different results in batches of inbred mice from the same supplier

2.1. Introduction

Different experimental results in rodents of the same strain have been reported pertaining to different suppliers (Lonjon et al., 2009, Palm et al., 2010). Researchers attribute these findings mainly to differences in genetic predisposition and genetic drift, which happens to occur when breeding rodents at different facilities over several years. We observe strong batch-related effects in the number of proliferating cells, young cells of neuronal lineage and total granule cells in equally-aged mice of the same supplier, indicating that even environmental factors potentially affect physiological processes that lead to different experimental results. Interestingly, we only see a baseline shift in cell parameters. No batch-related differences are found in the response of cell proliferation and AHN to experimental conditions and we do not find behavioral discrepancies in these animals. These findings lead to the suggestion that early life experiences affect physiological processes that determine the baseline number of neurogenesis but not the plasticity of neurogenesis to react to external stimuli.

2.2. Methods

2.2.1. Experimental procedure

We investigated two batches of reportedly 12-week old C57BL/6 mice (Charles River, Germany). The first batch was delivered and investigated twelve month before the second batch (batch 1: December 2009, batch 2: December 2010). Each batch contained 12 animals. After a quiet habituation of one week (see unpublished findings paragraph 1), animals were randomly assigned to either cages with (voluntary running group)- or without (control group) running wheel. Running wheels were connected to a controller system (AMS Software & Electronic GmbH, Flensburg, Germany), recording the performance of the mice in one-hour bins. Running mice had free access to their running wheels during the whole experimental period of two weeks. Data were collected once a day during the active period of the animals. Animals were weight before and after the experiment.

2.2.2. Perfusion

After two weeks, all animals were deeply anesthetized with pentobarbital before their bodies were perfused transcardially with cold phosphate buffered saline (PBS) and cold 4%

paraformaldehyde with 15% saturated picric acid (PFA). Brains were removed from the skull, hemispheres separated and postfixed overnight in PFA. Afterwards the right hemispheres were cryoprotected in sucrose, deep-frozen, cut into 40 µm sagittal sections and stored until further processing. Left hemispheres were dehydrated in ethanol (70% x 5 hours, 96% x 5 hours, 100% x 48 hours) before they were infiltrated with glycolmethylacrylate solution in four changes (24 hours, 3 days, 1 week, 1 week) (Technovit 7100, Kulzer GmbH, Wehrheim, Germany) and embedded according to manufacturer's instructions.

2.2.3. Immunohistochemistry for Ki67 and Doublecortin

Cell proliferation was visualized with an antibody-staining against the endogenous Ki67 nucleoprotein, which is expressed during the cell cycle (Starborg et al., 1996, Eisch and Mandyam, 2007). New cells of neuronal lineage were identified with an antibody-staining against the microtubule-associated protein Doublecortin (DCX) expressed during migration and growth of neuronal dendrites (Francis et al., 1999).

For Ki67 epitope retrieval, free floating 40 µm sagittal sections were incubated for 40 min in citrate buffer, pH 6.0, at 95°C. Afterwards, we preincubated sections in 2% normal goat serum (NGS), 0.1% bovine serum albumin (BSA) and 0.25% Triton in Tris-buffered saline (TBS), for 60 min at room temperature (RT). Incubation with the primary Ki67 antibody was performed overnight (polyclonal rabbit NCL-Ki67p, Novocastra, 1:5000 in preincubation solution). The following day sections were incubated for two hours with the secondary antibody (biotinylated goat anti-rabbit IgG 1:1000 + 2%NGS + 0.1%BSA in TBS) followed by incubation with a streptavidin-biotin complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) and with diaminobenzidine (DAB) as chromogen.

For DCX immunocytochemistry free floating 40 µm sagittal sections were incubated with 0.6% H_2O_2 in TBS-Triton to block endogenous peroxidase activity. Subsequently, sections were preincubated at RT with 2% normal rabbit serum (NRS), 0.1% BSA and 0.25% Triton in TBS and incubated overnight with the primary DCX antibody (polyclonal goat IgG, Santa Cruz Biotechnology, 1:1000 in preincubation solution) at 4°C. The incubation with secondary antibody (rabbit anti-goat IgG, Vectastain Elite ABS Kit, 1:1000 + 2%NRS + 0.1%BSA in TBS) was followed by incubations with a streptavidin-biotin-complex (Vectastain Elite ABC kit) and DAB. For counterstaining we used Ehrlich's haematoxylin.

All rinses before incubation with the primary antibody were accomplished in TBS-Triton, afterwards with TBS alone. Sections were mounted, dehydrated with alcohol, clearedand coverslipped.

2.2.4. Nuclear Nissl stain

Left hemispheres were cut into 20 µm horizontal sections. Every sixth section was mounted and dried for 1 hour at 60°C. Staining was accomplished with Giemsa solution (Merck, Darmstadt, Germany) diluted 1:10 in 0.07 M KH_2PO_4 buffer at RT for 1 hour. For differentiation the sections were incubated for 10 seconds in 1% acetic acid followed by 10 seconds in 96% ethanol. Finally, sections were dehydrated in two changes of absolute ethanol, cleared and coverslipped.

2.2.5. Counting

Total DCX numbers were estimated in every fifth section according to the optical fractionator principle (West et al., 1991) using the StereoInvestigator software (MicroBrightField Inc.Williston, USA). Cells were counted with a 100× oil-immersion lens (N.A.1.30) in a frame of 30 × 30 µm and with an x, y-step size of 125 µm between sampling locations. Cells in the top focal plane were not counted. Total cell numbers (N) were calculated with the formula $N = \Sigma Q^- \times (1/asf) \times 1/ssf$, ($Q^-$ = total number of cells counted, asf = area sampling fraction = a(frame)/a(x, y step) and ssf = section sampling fraction).

Ki67 positive-cells were counted manually in every fifth section using a 100× oil-immersion lens (N.A.1.30). Dying cells were identified by their strongly condensed C-or donut shaped chromatin bodies in the Giemsa-stained sections (Amrein et al., 2004a, Heine et al., 2004). Again, cells in the top focal plane of the section were excluded from counting. Total cell numbers (N) were calculated by multiplying the cell counts by the section sampling fraction (five).

Total granule cell numbers were estimated in every sixth glycolmethylacrylate-embedded Giemsa-stained section in control animals only. Cells were counted with a 100x oil-immersion objective (N.A. 1.30) according to the optical fractionator method (West et al., 1991) using the StereoInvestigator software (MicroBrightfield Inc.Williston, USA). A counting frame of 15 µm x 15 µm, a disector height of 10 µm and an x-y-steps size of 150 µm was used. Section thickness was measured at every 4th sampling site. Cell numbers were estimated using number-weighed section thickness.

2.2.6. Statistics

Statistical analyses were performed with the SPSS 19.0 statistical Software (SPSS Inc, Chicago, Illinois). One way ANOVA was used to determine batch-related differences in Ki67- and DCX-positive cells as well as total granule cell number. Differences in Ki67 and DCX between control- and running animals within batches were again elaborated with a one way ANOVA.

2.3. Results

2.3.1. Control animals

We found control animals of batch 1 and 2 to differ significantly in their number of Ki67- and DCX positive cells ($p_{Ki67}<0.001$, $p_{DCX}<0.001$; Fig. 7) as well as in the number of total granule cells ($p<0.001$; Fig. 8). The percentage differences in control animals between batches are 127% for Ki67, 54% for DCX and 44% for the total number of granule cells. Normalized to the total number of granule cells, control animals show a significant batch effect for Ki67 ($p=0.026$) but not for DCX ($p=0.517$).

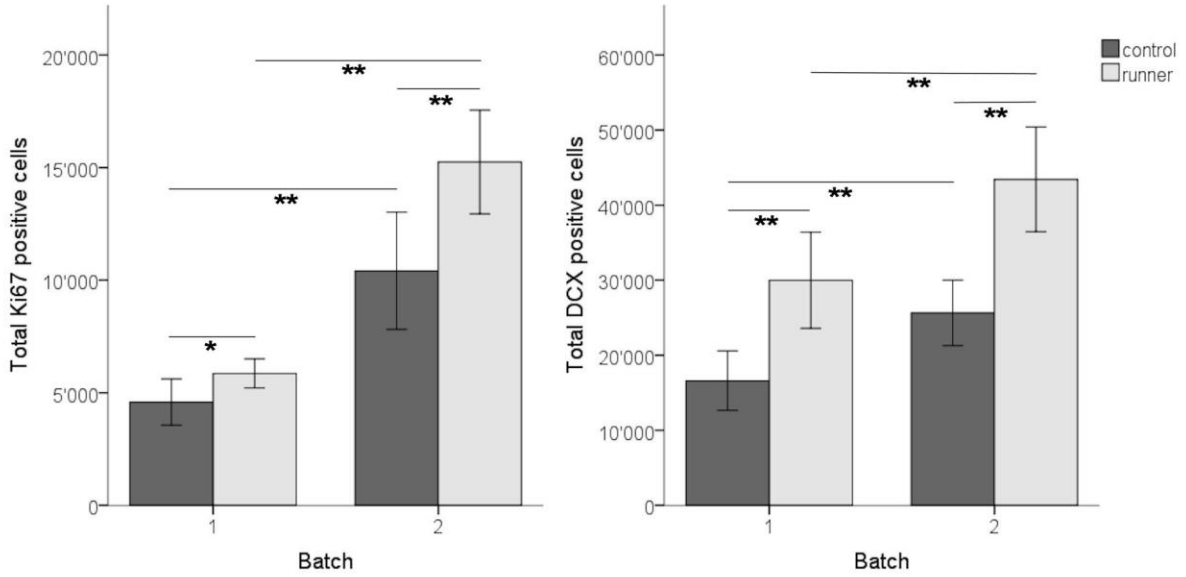

Fig. 7

Total numbers of Ki67- and DCX-positive cells of control and running animals of batch 1 (December 2009) and batch 2 (December 2010). **: $p<0.01$, *$p<0.05$. Bars = +/- 1 standard deviation.

2.3.2. Running animals

In both batches running induced a significant increase in the number of Ki67- and DCX-positive cells (batch 1: p_{Ki67}=0.033, p_{DCX}=0.003; batch 2: p_{Ki67}=0.005, p_{DCX}<0.001; Fig. 7). Running mice also display a significant batch effect (p<0.001) with percentage differences of 160% for Ki67 and 45% for DCX (Fig. 7).

No differences in activity patterns and performance levels could be detected between running animals of batch 1 and batch 2 (p=0.949; Fig. 9). Animals of the first batch performed on an average total level of 312'740 revolutions whereas mice of the second batch displayed an average total running activity of 314'207 revolutions (Fig. 9). In both batches average performances of the first week were significantly lower than average performances of the second week (batch 1: p<0.001; batch 2: p<0.001).

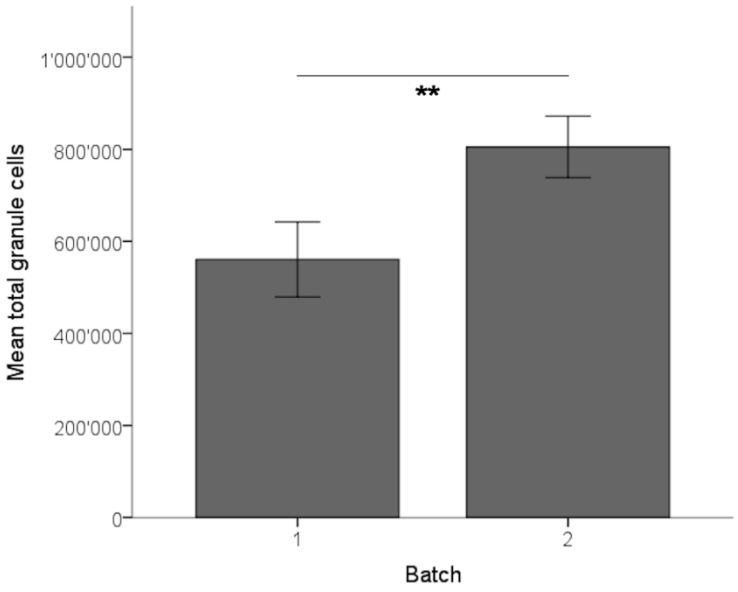

Fig. 8

Total number of granule cells in two batches of mice obtained from the same supplier in an interval of one year. **: p<0.01. Bars = +/- 1 standard deviation.

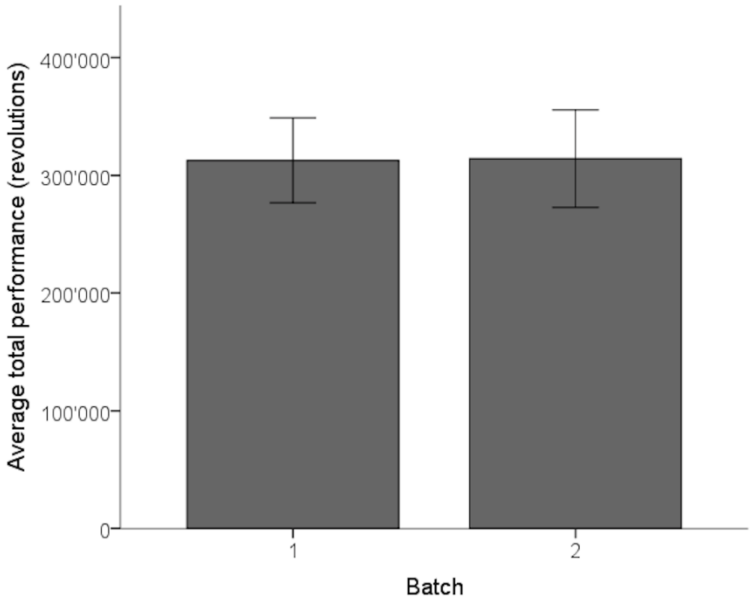

Fig. 9

Average total number of wheel revolutions in equally-aged mice of batch 1 and 2. Bars = +/- 1 standard deviation.

2.4. Conclusion

Animals of the same age and supplier which are investigated at different time points, i.e. delivered in separate batches, can show significant discrepancies in their number of proliferating cells, young neurons and total granule cells. Control groups as well as voluntary running animals display these batch-related differences, although no batch effect is found in the response of adult neurogenesis to running. Different batches have different baseline levels of cell proliferation and neuronal differentiation and the percent differences remain relatively constant after voluntary exercise. Interestingly, baseline differences in cell proliferation and neuronal differentiation do not affect the amount of running as all animals, which have free access to a running wheel, run about the same daily distance.

Until now it has been well accepted, that even in the same species minor genetic alterations can lead to molecular changes that potentially affect cellular processes such as adult neurogenesis. Mice of a different breed and origin can vary remarkably in their baseline level of AHN. In the present study, however, we exclude genetic influences as possible factors for the experimental differences because mice were tested in an interval of one year which seems a short period for substrain differentiation. Additionally, we found similar batch differences also in equally-aged C57BL/6 mice which were tested in a much shorter time interval of 4 weeks (data not shown). As already discussed in this thesis, influences such as stress, physical activity, enriched environment

or age have the potential to modulate adult neurogenesis in a short period of time. One could well imagine that small age-differences (if the supplier cheated a bit with the age of the animals), differences in social structure, cage environment or stress caused by transportation of the animals, led to changes in AHN. Modulatory effects, however, are unlikely to be responsible for the observed differences as they have only an effect on proliferating cells and young cells of neuronal lineage but not on the total number of granule cells (Ben Abdallah et al., 2010, Llorens-Martin et al., 2010). Additionally, weights of the animals did not indicate age-related differences. Curiously, here we find the main source for different adult neurogenesis levels in the number of hippocampal granule cells. Granule cell numbers show similar relative batch-differences than new cells of neuronal lineage, indicating that batch differences rather derive from long-term structural changes than from short-term modulatory mechanisms. The huge batch differences in cell proliferation, however, which exceed the differences in total granule cell number and young neurons, could very well derive from a short-term regulatory effect, as cell proliferation is detected in short time windows with the Ki67 antibody, while DCX expression of young neurons lasts about three weeks (Snyder et al., 2009a). Short-term effects cannot be fully elucidated as information about the numbers of dying- and glial cells would be essential for further conclusions. Short-term effects on cell proliferation, however, are of minor importance for the present study.

During Late prenatal and early postnatal period the vast majority of granule cells are formed in the dentate gyrus (Bayer, 1980). Because of the demonstrated tight relation between the number of granule cells and the number of young neurons, we believe that prenatal or early postnatal differences are also responsible for the determination of baseline rates of adult neurogenesis. Slomianka and colleagues found batch differences in the volume of the dentate gyrus, which are likely to be related to prenatal stress caused by the shipment of pregnant females (Slomianka et al., 1989). Additionally, different experiences during pregnancy, such as physical exercise, have been reported to influence AHN of the mouse offspring (Bick-Sander et al., 2006).

The findings of the present study question the easy comparability of results gained from different studies, even if animals have the same age and are derived from the same supplier.

3. Adult neurogenesis in the common marmoset (*Callithrix jacchus*)

3.1. Introduction

Adult neurogenesis has been reported in different rodent species as well as in higher order mammals, including primates (Gould et al., 1998, Gould et al., 1999b). However, only few studies about AHN in monkeys exist and very little is known about the quantity of newly formed cells in the dentate gyrus of different monkey species. We were given the opportunity to investigate adult neurogenesis in the common marmoset (*Callithrix jacchus*), which is a small new world monkey belonging to the family of the Callitrichidae. Investigating AHN of this small omnivorous monkey could provide an important insight into the regulatory and functional mechanisms of this phenomenon in higher order species and therefore contribute to the understanding of adult neurogenesis in the human condition as well.

We found cell proliferation in the common marmoset to be on a very low level compared to other mammal species in which cell proliferation in the adult stage has been investigated quantitatively. The picture gets even clearer when proliferation of different mammal species is related to the corresponding number of granule cells. In relation to the number of granule cells, the common marmoset, together with another primate species of the macaque family (*Macaca mulatta*), shows by far the lowest level of cell proliferation compared to other species. As for the number of newly generated neurons only qualitative observations are made so far. We find young cells of neuronal lineage in the hippocampal dentate gyrus, although the number of cells seems quite low compared to other species.

So far we can conclude that cell AHN in the common marmoset is present but to a very low extent. In view of the functional importance in contextual learning that has been attributed to the many thousands of young neurons that are generated in the dentate gyrus of laboratory rodents, it seems quite unrealistic that the few cells that we find in the common marmoset should provide the same functional properties. We propose that functional properties of AHN may have shifted during different lines of evolution, a conclusion also supported by the findings in bats which show very little or no adult neurogenesis at all despite of their need for high cognitive performance in spatial orientation.

3.2. Methods

3.2.1. Animals

For our study we used four adult common marmosets kindly provided by the Institute of Anthropology, University of Zurich. Animals needed to be sacrificed due to breeding control and animal husbandry of the primate facility. Three of the animals investigated were 2 years old and one 4 years old.

3.2.2. Perfusion

Animals were sacrificed with a mixture of Ketamin (10mg/kg) and Xylazin (0.5mg/kg) and post-mortally perfused with cold phosphate buffered saline (PBS) with Heparin (0.5ml/l), followed by 0.6% sodium sulphide solution in PB and 4% paraformaldehyde with 15% saturated picric acid (PFA). Brains were removed from the skull and separated sagitally into the two hemispheres and coronally into three blocks (frontal, medial, occipital). Postfixation was accomplished overnight in PFA. Afterwards the three blocks of the right hemisphere were cryoprotected in sucrose, deep-frozen, cut into 40 µm sagittal sections and stored in a cryoprotectant until further processing. The frontal, medial and occipital blocks of the left hemispheres were dehydrated in ethanol (70% x 5 hours, 96% x 5 hours, 100% x 48 hours) and infiltrated with glycolmethylacrylate solution in four changes (24 hours, 3 days, 1 week, 1 week) (Technovit 7100, Kulzer GmbH, Wehrheim, Germany). Embedding was done according to the manufacturer's instructions.

3.2.3. Stainings

Ki67 and DCX immunostaining of free floating sections as well as Giemsa nuclear stain of the glycolmethylacrylate sections were performed as described in paragraphs 2.2.3. and 2.2.4. of the unpublished findings-section.

3.2.4. Counting

Ki67-positive cells were counted manually on every tenth section with a 100× oil-immersion lens (N.A.1.30). Dying cells were identified by their strongly stained nuclei whose chromatin condensed into peripherally (C or doughnut shape), solid or multiple cell bodies (Amrein et al., 2004a, Heine et al., 2004). Cells in the top focal plane of the section were not counted. Total cell

numbers (N) were calculated by multiplying the cell counts by the section sampling fraction (ten).

Estimates of total granule cell numbers were made for the control animals of two batches in every twentyfifth glycolmethylacrylate-embedded Giemsa-stained section. Using the StereoInvestigator software (MicroBrightfield Inc.Williston, USA), total granule cell numbers were estimated with the optical fractionator method (West et al., 1991). Cells were counted with a 40x oil-immersion objective (N.A. 1.30) and a counting frame of 15 µm x 15 µm, a disector height of 10 µm and x, y-steps of 120 µm. Section thickness was measured at every 4th sampling site. Cell numbers were estimated using number-weighed section thickness.

3.3. Results

We estimated similar total numbers of Ki67 positive cells in all three animals aged two years ($Ki67_{animal\,1}$: 1'050 cells, $Ki67_{animal\,2}$: 1'460 cells, $Ki67_{animal\,3}$: 1'140 cells) but less cells in the animal that was four years old ($Ki67_{animal\,4}$: 350 cells). The average total number of Ki67 positive cells in the adult common marmosets is lower than in most mammals that we and others have investigated (Fig. 10). Compared to the yellow-necked wood mouse for example the average total number of Ki67-positive cells is in the common marmoset (*Apodemus sylvaticus*) is approximately five times lower, although the size of the hippocampus in a mouse is much smaller than that of a common marmoset.

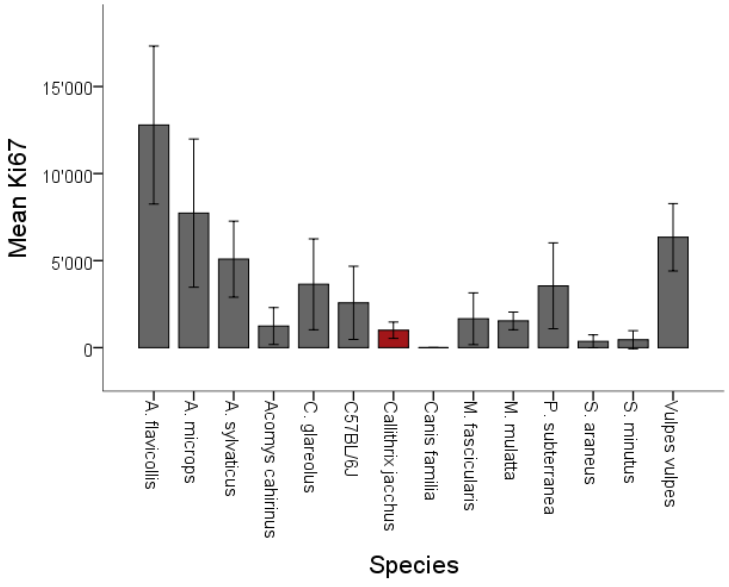

Fig. 10

Average total numbers of Ki67-positive cells in different mammalian species. Bars = +/- 1 standard deviation.

All data, exclusively data from *Macaca mulatta* (Gould et al., 1999b) and *Canis familiaris* (Siwak-Tapp et al., 2007), are derived from our laboratory.

Normalized to the number of granule cells, the percentage of proliferating cells in the common marmoset is on the lowest level compared to rodents (Fig. 11) sharing this lowest position with the rhesus monkey (*Macaca mulatta*) and the red fox (*Vulpes Vulpes*).

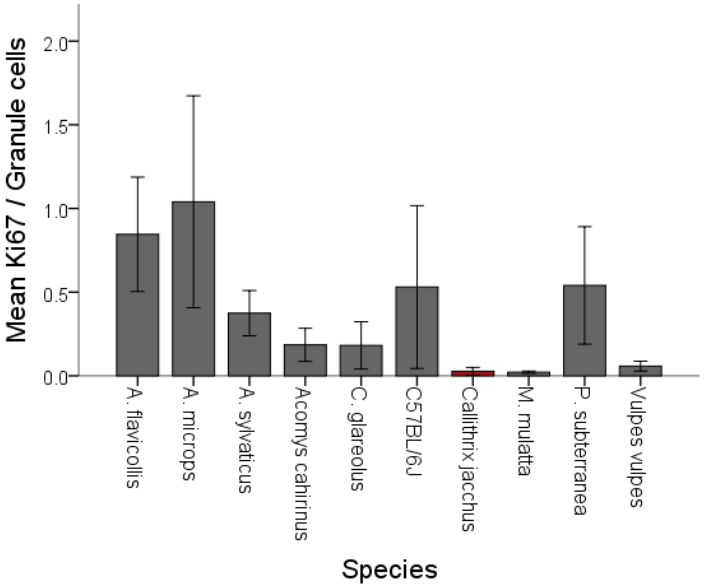

Fig. 11

Average number of Ki67-positive cells related to the number of total granule cells per hippocampus in different mammalian species. Bars = +/- 1 standard deviation.

All data, exclusively data from *Macaca mulatta* (Gould et al., 1999b, Keuker et al., 2003) and *Canis familiaris* (Siwak-Tapp et al., 2007), are derived from our laboratory.

Qualitative analysis of DCX-positive cells shows that young cells of neuronal lineage are formed in the subgranular layer of the hippocampal dentate gyrus. Distinct positively stained cell bodies can be identified in all regions of the dentate gyrus, although more numerous in the infrapyramidal blade. However, compared to DCX stainings of other species, for example C57BL/6- , wood mice or foxes, much less positively stained cells can be detected along the whole subgranular zone (Fig. 12).

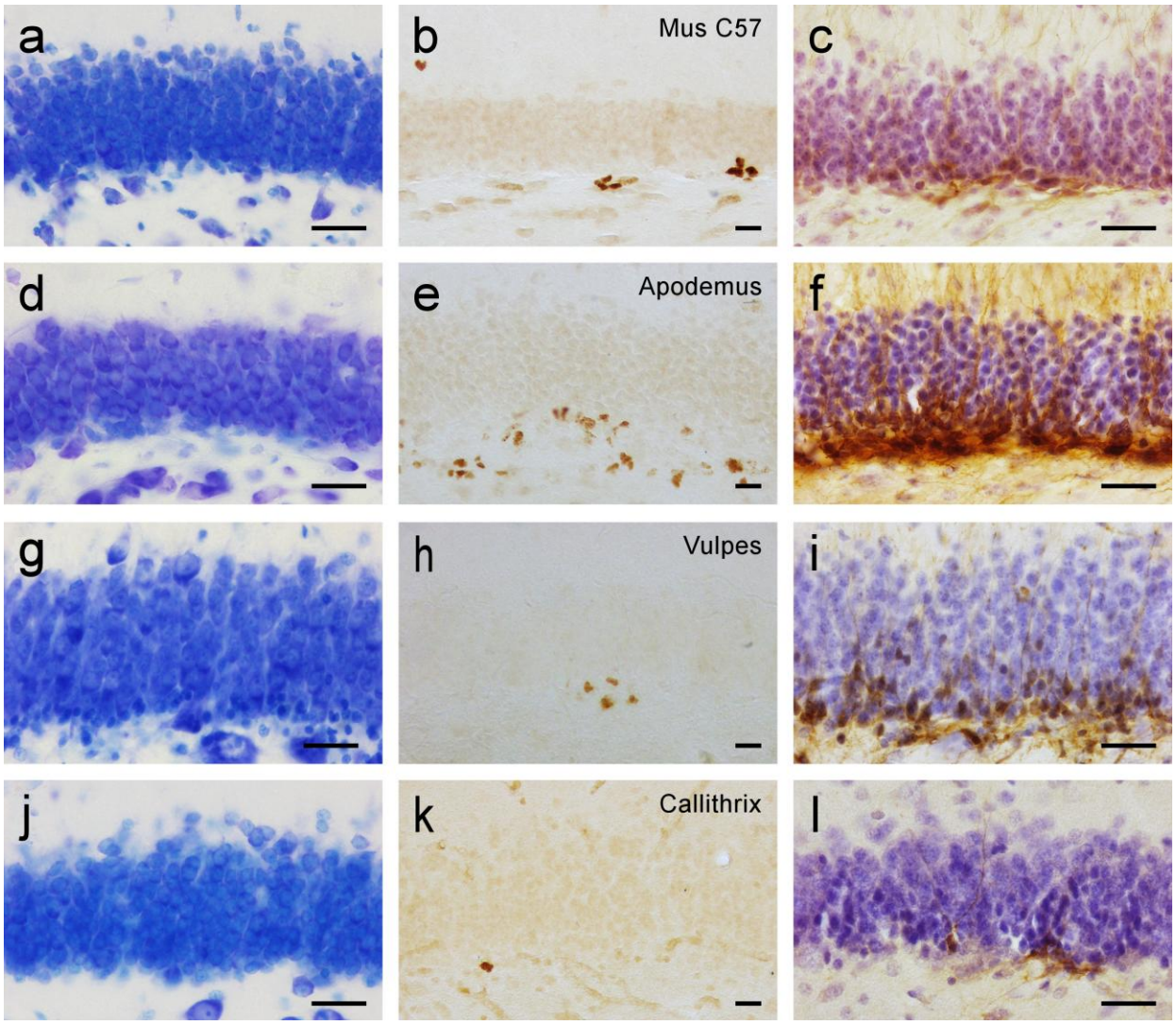

Fig. 12

Glycolmethylacrylate-embedded sections stained with Giemsa (a, d, g, j) and immunohistochemically stained sections for Ki67 (b,e,h,k) and DCX (c, f, i, l) in the laboratory C57BL/6- (a, b, c) and the yellow-necked wood mouse (*Apodemus flavicollis*) (d, e, f) as well as in the red fox (V*ulpes vulpes*) (g, h, i) and in the common marmoset (*Callithrix jacchus*) (j, k, l). Scale bars = 20µm.

3.4. Conclusion

We are able to confirm the presence of proliferating cells and young cells of neuronal lineage in the hippocampal subgranular zone in adult common marmoset monkeys (*Callithrix jacchus*). Number estimates for cell proliferation and qualitative analysis of young neurons, however, show that in the common marmoset adult neurogenesis occurs to a very low extent as compared to other species.

AHN in laboratory mice and rats has been functionally associated with cognitive functions, such as spatial memory and contextual learning. Numbers of newly generated neurons in the dentate gyrus of these animals have been shown to correlate with spatial learning performance in distinct spatial learning tasks. Extrapolating these findings to higher order species whose natural living conditions require also higher order cognitive abilities, one would expect adult neurogenesis in these animals to be on a higher level. At least AHN relative to the size of the corresponding hippocampus or to the number of granule cells would be expected on an equal level than it is in smaller mammals. Cognitive skills in contextual tasks seem quite well developed in the common marmoset. Even if not entirely proved, individuals are thought to socially transmit information via active imitation (Caldwell and Whiten, 2004). Somewhat surprisingly, in the common marmoset and in most higher order species that have been investigated so far, the numbers of proliferating and neuronal differentiating cells are far lower than in rodents. The rhesus monkey, which shares 93% of its genome with humans (Gibbs et al., 2007) and is thought to have high developed cognitive skills, shows, as the common marmoset, a comparably low level of cell proliferation and neurogenesis (Jabes et al., 2010). The number of neurogenic cells that has been found in humans is even below every investigated mammalian species (Eriksson et al., 1998). These findings indicate that the functional significance of adult neurogenesis in higher order animals should be considered rather critically. As for cognitive abilities in spatial orientation and contextual learning it seems quite unrealistic that in more encephalized species a relatively much lower number of young neurons should provide superior cognitive abilities.

Further examples for animals, in which one would expect neurogenesis to play a crucial role for spatial orientation are bats. Bats have well-developed 3-D navigation abilities. They cover large territories and some bat species are even known for long-distance migratory behavior. Interestingly, in most bat species hippocampal neurogenesis during adulthood is absent. Only in a few number of species a few cells have been detected (Amrein et al., 2007).

Taken together, comparative results pertaining to adult neurogenesis in different mammalian species indicate that functional aspects of these newly generated cells in the hippocampus may differ between species. The functional significance of AHN in cognition and memory could have shifted during different directions of evolution. In different evolutionary lines the cognitive development may have led to an increased complexity in brain processing whose requirements may have exceeded the potential of adult neurogenesis.

PUBLICATIONS

1. **Hauser T, Klaus F, Lipp HP, Amrein I** (No effect of running and laboratory housing on adult hippocampal neurogenesis in wild caught long-tailed wood mouse. BMC Neurosci 10:43.2009).

2. **Klaus F, Hauser T, Slomianka L, Lipp HP, Amrein I** (A reward increases running-wheel performance without changing cell proliferation, neuronal differentiation or cell death in the dentate gyrus of C57BL/6 mice. Behav Brain Res 204:175-181.2009).

3. **Klaus F, Hauser T, Lindholm AK, Cameron HA, Slomianka L, Lipp HP, Amrein I** (Different regulation of adult hippocampal neurogenesis in Western house mice (Mus musculus domesticus) and C57BL/6 mice. Behav Brain Res: reviewed manuscript).

4. **Klaus F, Amrein I** (Running in laboratory and wild rodents: Differences in context sensitivity and plasticity of hippocampal neurogenesis. Behav Brain Res: accepted manuscript).

DISCUSSION

Summarizing the outcomes of the different studies that have been conducted during this thesis, we were able to show that AHN and behavioral wheel-performance in laboratory mice are dependent on external factors (Klaus et al., 2009, Klaus et al., 2011 and Unpublished findings). We showed that the baseline level of adult neurogenesis in laboratory animals can vary in equally-aged animals of the same laboratory strain (see Unpublished findings). Additionally, neurogenesis in laboratory mice can be increased by voluntary physical exercise but the positive effect of running is lost as soon as the motivational context changes from a voluntary to a forced condition (Klaus et al., 2011) or to a condition, in which the mice are motivated to run more than they would do voluntarily (Klaus et al., 2009). Performance levels can vary considerably depending on the housing environment (see Unpublished findings).

Further, the results of our studies indicate that AHN in laboratory mice is regulated differently as compared to closely related wild mouse species like the long-tailed wood mouse (*Apodemus sylvaticus*) (Hauser et al., 2009) or the western house mouse (*Mus musculus domesticus*) (Klaus et al., 2011). Wild mice seem to have a rather stable rate of adult neurogenesis that does neither react to running under different motivational contexts nor to impoverished or stress-related situations. A common finding across all running studies and all investigated mouse species, however, is that the individual level of running activity does not correlate with individual AHN (Hauser et al., 2009, Klaus et al., 2009, Klaus et al., 2011).

Results of a side project, which is not directly related to the main topic of the thesis, revealed adult neurogenesis in the common marmosets (*Callithrix jacchus*) to be on a very low level compared to other mammalian species (see Unpublished findings).

In the following sections these findings will be discussed in more detail by trying to fit them all together into a coherent picture, in which modulatory as well as functional aspects of AHN will be considered on a species-specific level.

1. Plasticity of hippocampal neurogenesis - natural phenomenon or domestication effect?

Many studies highlight the enormous plasticity of AHN in laboratory animals in response to external influences like voluntary exercise (summarized in the Introduction section). Indeed, we are able to confirm the positive response of adult neurogenesis to running in laboratory mice (Klaus et al., 2009, Klaus et al., 2011), yet our results reveal that such modulation is limited. The positive effect of running seems to strictly depend on a voluntary motivation. Rewarding the mice for their performance leads to an increased performance but this increase does not translate into an additive positive response in adult neurogenesis (Klaus et al., 2009). Additionally, cell proliferation remains at control levels and the number of young neurons is significantly lower than in voluntary running controls when mice have to run for their daily need of food (Klaus et al., 2011). The influence of the contextual background is confirmed by studies which show that forced treadmill-running above a certain speed-level is no longer beneficial for adult neurogenesis (Kim et al., 2003). Further, social stress in laboratory rats prevents the positive effect of short term exercise on AHN (Stranahan et al., 2006). In laboratory rodents it seems that the combination of external stimuli decreases the plasticity of adult neurogenesis. One could very well imagine that with an increasing number of external stimuli or an increasing complexity of the environment, positive and negative effectors on adult neurogenesis balance each other decreasing the modulatory power of each stimulus, thus leading to a relatively stable adult neurogenesis.

For translating rodent results regarding external modulation of adult neurogenesis to the human condition, the most important questions are (i) whether and to what extent adult neurogenesis can be modulated under natural conditions, and (ii) whether and how the modulation differs between species. Mimicking a naturalistic situation for laboratory mice in which they have to run for food, i.e. food achievement is combined with a working investment, reveals a significant decrease in the plasticity of AHN (Klaus et al., 2011). Creating naturalistic situations in a laboratory setting using laboratory mice, however, can only provide us limited insight into the real mechanisms behind adult neurogenesis. Only limited numbers of external stimuli can be combined in a laboratory. Moreover, physiological processes in genetically homogenous inbred strains are unlikely to fully represent the processes happening in genetically heterogeneous wild species possessing full homeostatic capacities.

Going one step into the direction of understanding naturally occurring processes pertaining to adult neurogenesis, we investigated wild or wild-derived mouse species in the same running paradigms as laboratory mice (Hauser et al., 2009, Klaus et al., 2011). With this experimental setup not all requirements of natural regulation of AHN are fulfilled as animals are investigated under laboratory conditions, but it allows the direct comparison of regulatory mechanisms between laboratory- and wild or wild-derived mice. Working with very closely related species minimizes strong genetic effects. Thus, we tested wild-caught wood mice (*Apodemus sylvaticus*) which are closely related to the common laboratory mouse strains (Michaux et al., 2002, Steppan et al., 2005), and F1 offspring of wild-derived western house mice (*Mus musculus domesticus*) which are the closest relatives of laboratory mice, sharing 92% of their genome with the C57BL/6 mouse (Yang et al., 2007). Laboratory- and wild-derived mice differ significantly in adult neurogenesis after voluntary running. Also, running under a forced context (Klaus et al., 2011), under a stressing situation or under the lack of environmental complexity (induced by introducing wild mice into laboratory conditions) have no significant modulatory effect on the number of dividing or neuronally differentiating cells (Hauser et al., 2009). In summary, AHN in wild or wild-derived mice of the investigated species differs considerably from what we find in laboratory mice. The results imply that the genetic setup is more crucial in determining the potential of adult neurogenesis to react to external stimuli than the environmental stimulus itself.

Clearly, laboratory mice tested under more naturalistic conditions, i.e. experiencing not only one stimulus which potentially affects AHN but a combination thereof, show no or weak plasticity of adult neurogenesis, which resembles the findings in wild mice. Thus, the difference in regulation as observed in laboratory mice appears to be linked to an exaggerated response to a single stimulus in absence of other inputs. We believe that the constant exposure to different stimuli that potentially affect adult neurogenesis (see Introduction) has led to a natural selection which stabilizes AHN in the wild. In contrast, during domestication, a loss of homeostatic regulation appears to have occurred, resulting in relatively uncontrolled reactivity to single stimuli occurring in a stimulus-poor environment. Domestication generally entails a relaxation from many selective pressures operating on morphology and physiology, but can include artificial selective breeding for traits (e.g., fear responses, coat colors, all-season reproduction) that would impair biological fitness in the wild. For example, domestication often leads to a reduction in size of brains and

sensory organs (Kruska, 2005). Pertaining to AHN, domestication appears to have entailed loss of regulatory pathways operative in wild animals.

Our findings imply that extrapolating from laboratory mice to humans is more complicated than envisioned initially. The environmental complexity humans experience is rather comparable to wild-living than laboratory animals, unless one assumes that the modern Homo sapiens is undergoing domestication. Differences not only on a modulatory background but also at the species level complicate the picture.

2. Functional relevance of adult neurogenesis - species-specific differences in functional requirements

The findings that species differ considerably in their level of adult neurogenesis and that plasticity of AHN depends on species as well as on domestication raise the question, if these differences are also reflected in functional discrepancies. The huge difference in adult neurogenesis found between rodent species and the common marmoset implies that functional requirements of AHN do differ between species.

As already discussed in the Introduction section, the function of newly generated granule cells is still an issue of debate. However, evidence has accumulated that adult neurogenesis is at least partly involved in hippocampus-dependent cognitive skills such as spatial memory formation and contextual learning (see Introduction). This association is not surprising in view of the fact that granule cells of the dentate gyrus are involved in the formation of spatial maps- also in association with different contexts (see Introduction). During an animal life thousands of new declarative memories are formed. The hippocampus is therefore subject to constant inputs and structural changes requiring a high level of neuronal plasticity. The generation of new cells of the neuronal lineage in the subgranular zone of the dentate gyrus could contribute to the high plasticity required for the continuous formation of new declarative memories.

However, only in laboratory rodents we find AHN to be plastic enough to react quantitatively to external changes. Neurogenic plasticity in laboratory animals seems to work from two sides. First, adult neurogenesis is increased in response to a learning process. Spatial learning (Gould et al., 1999a) and exploring an enriched environment (Kempermann et al., 1997b) have been shown to increase the number of new cells of neuronal lineage by increasing the survival of young neurons that have been generated before but not during learning. In these situations increasing numbers of young granule cells could well be associated with the formation of new contextual memories related to space. Second, new cells are generated before they can be recruited to form new memories. In this case an external stimulus like exercise, which is not related to spatial memory, increases cell proliferation and thereby also the percentage of young neurons differentiated. Running mice which are introduced into a learning situation have more young cells that can potentially be recruited to generate new memories and therefore show a better learning performance than non-running mice (van Praag et al., 1999a, van Praag et al., 2005,

Creer et al., 2006). During the learning process itself survival of the existing pool of young neurons could be promoted. Reports about correlations between exercise, adult neurogenesis and cognitive skills are yet contradictory and associations seem, amongst others, to depend on the genetic background of the animals (see Introduction). However, plasticity on the level of changing numbers of proliferating and differentiating cells in the case of laboratory rodents could very well account for different requirements in memory processing but this theory only holds true for domesticated mice. We were able to show that AHN in wild mice remains relatively stable in spite of external influences that induce changes in adult neurogenesis of laboratory animals (Hauser et al., 2009, Klaus et al., 2011). The formation of new memories seems therefore not to be associated with increased numbers of newly generated cells. Plasticity in cell proliferation and neuronal differentiation is costly and time consuming and may be an appropriate way to functionally react to environmental changes in the impoverished environment of a laboratory but maybe not to fulfill the requirements in a much more complex and constantly changing environment. Plasticity required to form new memories in a natural environment may therefore rather occur via the reorganization of the persisting neuronal network on the level of dendritic arborization or synapse formation. Thus, new neurons in wild animals may help to maintain the functional aspects of the highly challenged hippocampal structure by replacing dying granule cells or could simply act as a distinct population with a completely different function. The fact that in some higher order species, like in the common marmoset, cell proliferation and neuronal differentiation only happens to a minor extent and that in other species AHN is not required during all (Amrein et al., 2007) or part (Bartkowska et al., 2008) of the adult life, confirms that neuronal plasticity accompanying memory formation does not necessarily depend on adult neurogenesis.

Whatever functional explanation for the phenomenon of AHN might apply, one is left to ask why there is a very rapid age-dependent decline in the basal proliferation level.

Again these conclusions highlight the difficulty to extrapolate from one species to another. Functional aspects of adult neurogenesis may be similar comparing two species but completely different comparing two other species.

3. The association between performance and adult hippocampal neurogenesis

"The more you run the more neurons you have the cleverer you are". According to the plasticity of AHN in response to physical exercise and the subsequent possible benefit for cognitive performance, this intuitive assumption became imprinted in people's minds. A few research groups report a correlation between the activity level of laboratory animals and the number of young neurons that are being formed in the subgranular zone (Allen et al., 2001, Bednarczyk et al., 2009) but we were not able to confirm this result in any of our experiments.

The most consistent finding across all our studies is that individual voluntary performance in a running wheel does not correlate with the number of proliferating cells or cells of neuronal lineage (Hauser et al., 2009, Klaus et al., 2009, Klaus and Amrein, 2011, Klaus et al., 2011). For laboratory as well as for wild mice we show, that under voluntary free wheel-access conditions low activity levels do not correspond to low rates of adult neurogenesis and that comparably high levels of wheel activity do not predict high AHN. The lack of a correlation gets even more apparent when considering that equally-aged animals of the same strain are very consitstent in their performance but differ extremely in their level of adult neurogenesis (see Unpublished findings). This is coherent with the lack of a genetic correlation between levels of adult neurogenesis and activity in recombinant inbred strains (Kempermann and Gage, 2002a).

This conclusion applies also to other mouse species. Wild wood mice have a very wide range of activity but a very tightly regulated AHN that does not correspond to the variability of their performance (Hauser et al., 2009, Klaus and Amrein, 2011). Wild-derived house mice, which show the highest average running performance, have a lower average level of adult neurogenesis than C57BL/6 mice (Klaus and Amrein, 2011). To go one step further, the theory of activity levels predicting the number of neurogenic cells does not hold within ((Klaus and Amrein, 2011) and between taxonomic groups as highly active shrews (Bartkowska et al., 2008) and several bat species (Amrein et al., 2007) do not show adult neurogenesis at all but voles, known to be relatively lazy covering only small territories, show AHN during adulthood (Amrein et al., 2004a).

We conclude that both, activity differences in the wild and variations of voluntary wheel activity in wild animals, have no significant influence on baseline levels of adult neurogenesis. This is not further surprising because, as already mentioned, we believe that AHN in wild animals is set to a

species-specific stable level which is not likely to be influenced by environmental differences or external influences. More surprising is the fact that in laboratory mice differences in the level of wheel activity do not lead to corresponding discrepancies in adult neurogenesis as the number of young neurons seems rather plastic. Indeed AHN is up-regulated by exercise but the extent to which this process happens depends rather on the fact that the animal has the possibility to be active at all than on the fact how much it runs. Big differences in running behavior in laboratory mice can be experimentally induced after changing the motivation to run by rewarding the performance (Klaus et al. 2009), or when testing the animals in a stressful environment (see Unpublished findings). Unfortunately, we did not investigate adult neurogenesis in the latter case. Nonetheless, a 78% increase in performance in the rewarded situation that does not translate into a corresponding increase in AHN is strong evidence for a lack of correlation between performance and adult neurogenesis. In theory, voluntary running in laboratory mice could be sufficient to up-regulate adult neurogenesis to a ceiling level which cannot be further changed, thus masking an existing correlation. Restricting access to a running wheel could provide valuable information about an existing correlation at lower activity levels.

3. Final conclusions

The main statements that can be made out of all our studies are that no- or only weak relation exists between running and AHN in wild- and wild-derived mice and that also the running-induced regulation of adult neurogenesis is limited in C57BL/6 mice depending on context of the running situation. Domestication may have entailed an over-reactivity to single stimuli or might have promoted abnormal regulatory mechanisms. A lack of a general correlation between running and AHN is supported by the missing genetic correlations between AHN and running levels in recombinant inbred lines and by the absence of AHN in highly active species. Thus, the simplistic translation of a direct relationship between exercise, AHN and cognition to the human condition seems intuitively appealing but is neither supported by rodent- nor comparative studies.

ABSTRACTS OF POSTER PRESENTATIONS

1. **Klaus F., Slomianka L., Amrein I., Lipp H.-P.** (2008). Influence of reward on exercise performance and adult hippocampal neurogenesis in C57BL/6 mice. ZNZ Symposium, Zurich, Switzerland.
2. **Hauser T., Klaus F., Slomianka L., Amrein I., Lipp H.-P.** (2008). The effect of running on cell proliferation and neurogenesis in the hippocampus of long tailed wood mice (*Apodemus sylvaticus*). FENS, Geneva, Switzerland, Switzerland.
3. **Klaus F, Hauser T, Slomianka L, Amrein I, Lipp H-P.** (2009). Does running for food have a stress-related impact on adult hippocampal neurogenesis in C57BL/6 mice? NCCR "Neural Plasticity and Repair", Berlingen, Switzerland.
4. **Klaus F., Hauser T., Lipp H.-P., Amrein I.** (2009). Regulatory mechanisms of adult hippocampal neurogenesis in laboratory and wild wood mice: differential response to physical exercise. ZNZ Symposium, Zurich, Switzerland.
5. **Klaus F., Hauser T., Slomianka L., Lipp H.-P., Amrein I.** (2009). Physical exercise effects adult hippocampal neurogenesis differently in laboratory and wild wood mice (Apodemus sylvaticus). International SfN (Society for neuroscience) congress, Chicago, USA.
6. **Klaus F., Slomianka L., Lipp H.-P., Amrein I.** (2010). Psychological backgrounds determine running-induced modulation of neurogenesis. Adult Neurogenesis: Structure and Function, Frauenchiemsee, Germany.
7. **Klaus F., Slomianka L., Lipp H.-P., Amrein I.** (2010). Diverse motivational backgrounds of wheel running affect neurogenesis differently. FENS, Amsterdam, Netherlands.
8. **Klaus F., Nötzli1 S., Berry A., Cirulli F., Giorgio M., Pelicci P.G., Lipp H.-P., Amrein I.** (2010). Sustained pool of hippocampal neurogenic precursor cells in senescent p66Shc-/- mice. ZNZ Symposium, Zurich, Switzerland.
9. **Klaus F., Hauser T., Lindholm A.K., Cameron H.A., Slomianka L., Lipp H.-P., Amrein I.** (2011). Different regulation of adult hippocampal neurogenesis in Western house mice (Mus musculus domesticus) and C57BL/6 mice. NCCR "Neural Plasticity and Repair", Ittingen, Switzerland

1. Influence of reward on exercise performance and adult hippocampal neurogenesis in C57BL/6 mice

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Institute of Anatomy, University of Zurich, Switzerland

This study investigated whether a reward affects the individual performance and the rate of cell proliferation and neurogenesis in C57BL/6 mice. Over an experimental period of 2 weeks 24 animals were housed individually either in cages containing a running wheel or in cages without any environmental enrichment. Half of the running cages were connected to a reward system during the second week of the experiment whereby the mice were rewarded for a predetermined number of wheel revolutions. The performance of each mouse was recorded in one hour bins. After the exercise experiment mice were perfused and their brains immunohistochemically stained for the cell proliferation marker Ki67 and for the neuronal lineage marker doublecortin (DCX)

Reward significantly increased the individual performance during the second week of the experiment compared to the non-rewarded performance of the first week, whereas the performance of the voluntary runners remained relatively constant during the whole experiment.

Running in general was able to significantly increase cell proliferation and neurogenesis but reward and the associated increase of performance did not have an additional modulatory effect on proliferation and the number of cells entering the neuronal lineage.

Supported by SNF and NCCR “Neural Plasticity and Repair” and INTELLIMAZE 037965

2. The effect of running on cell proliferation and neurogenesis in the hippocampus of long tailed wood mice (*Apodemus sylvaticus*).

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Adult hippocampal cell proliferation and neurogenesis in wild rodents proceed on a species-specific level. Unknown are modulators of neurogenesis for these animals. Given the fact that physical exercise leads to an increase of neurogenesis in laboratory mice, this study investigates the effect of running on neurogenesis in the long tailed wood mice (*Apodemus sylvaticus*).
20 wild caught long tailed wood mice were housed individually in cages containing a running wheel and no environmental enrichment. The running data, that were registered daily, revealed a high variability in the running performance among the mice. Three groups of mice with similar running performance can be distinguished.

Cell proliferation and neurogenesis were visualized immunohistochemically with the markers for proliferating cells (Ki67) and neuronal progenitors/young neurons (doublecortin). Data of six long tailed wood mice perfused immediately after trapping serve as baseline data and were compared to the data of runners and non-runners. Analyses show that running has no effect on the rate of neurogenesis or on the rate of cell proliferation. In addition there exists no relation between interindividual differences in the levels of cell proliferation and neurogenesis and interindividual differences in the level of voluntary running. Furthermore impoverished environment has no effect on the rate of cell proliferation and neurogenesis.

Supported by SNF and NCCR "Neural Plasticity and Repair" and INTELLIMAZE 037965

3. Does running for food have a stress-related impact on adult hippocampal neurogenesis in C57BL/6 mice?

F. Klaus, T. Hauser, L. Slomianka, I. Amrein, H.-P. Lipp,
Institute of Anatomy, University of Zurich, Switzerland

Previous data have shown that C57BL/6 mice rewarded for their performance increase their running level almost 80%. This increase in performance does not result in an enhanced neurogenesis compared to voluntary running animals. We suggest that running in a rewarded context causes a stressing situation where an enhancing effect of running is counterbalanced by a negative psychological effect. In the present setup we test whether running for the daily need of chow causes any changes in neurogenesis which can be related to a psychological stress.

Running for the daily need of food causes a decrease in neurogenesis to levels which are no more statistically different from control levels. Given the facts that both running groups performed at almost precisely the same level and that animals were not food deprived, we suggest that running for food causes a psychological stress related decrease in neurogenesis or that stereotypic “task” performance down regulated adult hippocampal neurogenesis.

Supported SNF and NCCR “Neural Plasticity and Repair”

4. Regulatory mechanisms of adult hippocampal neurogenesis in laboratory and wild wood mice: differential response to physical exercise

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Adult hippocampal neurogenesis (AHN) of laboratory mice is a plastic process which can be up- and down-regulated by different internal and external factors. A strong positive modulation of AHN through physical exercise in well documented for laboratory rodents. This study compares the regulatory effect of running in laboratory C57BL/6 and wild-caught wood mice (Apodemus sylvaticus). While the wild animals are tested in standard voluntary wheel running conditions, running of laboratory mice is combined with a reward. Interestingly, we find no exercise related changes in cell proliferation and neuronal differentiation in the wild wood mice. Rewarded C57BL/6 mice display an increase in performance of 78% which does not translate into changed levels of AHN. Individual performance and neurogenesis does not correlate in C57BL/6 and wood mice. On group level however, the resistance of AHN in wild wood mice to exercise and environmental changes contrasts to the high plasticity of this process in C57 mice, where we see a strong dependence of neurogenesis on exercise but not on the performance level of the animals. We show that the regulatory mechanism of neurogenesis differs between these two species, and that the exercise-induced modulation of AHN in laboratory animals is limited.

Supported by SNF and NCCR "Neural Plasticity and Repair" and INTELLIMAZE 037965

5. Physical exercise effects adult hippocampal neurogenesis differently in laboratory and wild wood mice (*Apodemus sylvaticus*)

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Adult hippocampal neurogenesis (AHN) of laboratory mice is a plastic process which is up- and down-regulated by different internal and external factors. Modulation of AHN through physical exercise in a running wheel has been investigated by many research groups, highlighting a strong increase of cell proliferation accompanied by an increase in the number of newly generated neurons. This study focuses on identifying the range in which AHN can be manipulated in laboratory C57BL/6 and wild-caught wood mice (*Apodemus sylvaticus*). While the wild animals are tested in standard voluntary wheel running conditions, the running of the laboratory mice is combined with a reward stimulus. Interestingly, we find no exercise related changes in cell proliferation and neuronal differentiation in the wood mice. Rewarded C57BL/6 mice display an increase in performance of 78% which does not translate into changed levels of cell proliferation and neurogenesis. Individual performance and neurogenesis do not correlate in C57BL/6 or wood mice. On species level, the resistance of AHN in wild wood mice to exercise and environmental changes contrasts with the high plasticity of this process in C57 mice, where we see a strong dependence of neurogenesis on exercise but not on the performance level of the animals. Though the regulatory mechanism of neurogenesis is different in these two species, it seems clear that also the exercise-induced modulation of AHN in laboratory animals is limited.

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6. Psychological backgrounds determine running-induced modulation of neurogenesis

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Adult hippocampal neurogenesis can be modulated positively or negatively by many external and internal factors. Voluntary running is one of the most investigated positive stimulators of hippocampal neurogenesis whereas stress has been shown to influence the system negatively. In the present study we investigate cell proliferation, differentiation and cell death in the hippocampus of C57BL/6 mice running under different psychological conditions. Voluntary running mice are compared to animals which are chocolate-rewarded for their performance and to animals that have to run for their daily need of chow. All running groups have free access to the running wheel over the whole experimental period of two weeks and their performance is detected with a controller system recording the number of revolutions in one hour bins. Cell proliferation is immunohistochemically visualized with an antibody against Ki67 protein and differentiating cells of neuronal lineage with an antibody against the microtubule-associated protein Doublecortin (DCX). Pyknotic cells are used as indicators of neuronal death.
Voluntary running and rewarded running animals display a significant increase in cell proliferation and neuronal differentiation compared to control animals. But despite a substantial increase in performance of the rewarded runners compared to voluntary runners, neurogenesis levels do not differ from each other. Interestingly, mice that need to run for their food perform on the same level than the voluntary runners but display significantly less proliferating and neurogenic cells and an enhanced number of pyknotic cells.

We conclude that the beneficial effect of running on neurogenesis depends on the emotional valence of the running situation. As long as running is performed on a positive motivational background it has a positive effect on neurogenesis, which seems to be independent of the performance level, but as soon as running is combined with a psychological stress situation it loses this beneficial effect.

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7. Diverse motivational backgrounds of wheel running affect neurogenesis differently

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Voluntary exercise in a running wheel is one of the best known stimulators of adult hippocampal neurogenesis in laboratory rodents. In the present study we investigate cell proliferation, differentiation and cell death of C57BL/6 mice being subjected to different motivational running backgrounds. Voluntary running mice are compared to animals which are chocolate-rewarded for their performance and to animals that have to run for their daily need of chow. All running groups have free access to the running wheel over the whole experimental period of two weeks and their performance is detected with a controller system recording the number of revolutions in one hour bins. Proliferating cells are immunohistochemically stained for Ki67 and differentiating cells of neuronal lineage for the microtubule-associated protein Doublecortin (DCX). Pyknotic cells are used as indicators of neuronal death.
Both, voluntary running and rewarded running animals display a significant increase in cell proliferation and neuronal differentiation compared to control animals. But despite a 78% increase in performance of the rewarded runners compared to voluntary runners neurogenesis levels do not differ from each other. Interestingly mice which need to run for their food perform on the same level than voluntary running animals but do not show a significant increase in neurogenic markers compared to controls.

We conclude that the beneficial effect of running on neurogenesis depends on the emotional valence of the experimental condition. As long as running is performed on a positive motivational background it has an enhancing effect on neurogenesis, which seems to be independent of the performance level, but as soon as running is combined with a psychological stress situation it loses this beneficial effect.

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8. Sustained pool of hippocampal neurogenic precursor cells in senescent p66Shc-/- mice

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P66Shc-/- mice show increased resistance to oxidative stress and lifespan extension of ~ 30%. The protein, a splice variant encoded by the proto-oncogene locus SHC, is involved in the transmission of mitogenic signals from activated receptors to the proto-oncogene Ras. Mutants appear more resistant against cardio-vascular degeneration, show reduced age-dependent emotionality and pain-sensitivity, better behavioral plasticity in a spatial memory task and increased levels of neurotrophin BDNF. We investigate old (21-24 months) p66Shc-/- (KO) and p66Shc+/+ (WT) littermates for proliferation, neuronal differentiation and cell death in the hippocampus. We find equal numbers of dying cells in KO and WT. Proliferation is significantly increased in p66Shc-/- mice with a strong genotype*gender interaction in favor of females. We find that all females (KO and WT) have significantly more young neurons. Our data indicate a sustained number of granule cell precursors in the dentate gyrus of senescent KO mice compared to WT, indicating increased resistance to oxidative stress in hippocampal precursor cells as shown for other cells in KO mice. Other studies have shown that in senescence, neurogenesis-stimulating factors target mostly survival. In our study, cell survival particularly in females seems to be receptive for any stimulus, an observation that needs further investigation.

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9. Different regulation of adult hippocampal neurogenesis in Western house mice (*Mus musculus domesticus*) and C57BL/6 mice

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Adult hippocampal neurogenesis (AHN) of laboratory rodents is enhanced by physical exercise in a running wheel. However, little is known about modulation of AHN in wild-living rodent species. The finding that AHN cannot be modulated by voluntary exercise in wild wood mice suggests that AHN may be regulated differently under natural conditions than in laboratory adapted animals. To further investigate this difference, while minimizing genetic influences, we investigated C57BL/6 mice and their genetically closest wild-living relatives under the same experimental conditions. C57BL/6 mice and F1 offspring of wild house mice (Mus musculus domesticus) were tested in two different running paradigms: voluntary running and running-for-food - a condition in which mice had to run for their daily allowance of food. In house mice, we found a non-significant trend towards increased numbers of proliferating cells and young cells of neuronal lineage in both voluntary runners and runners-for-food. Voluntary running in C57BL/6 mice resulted in a 30% increase in cell proliferation and a pronounced 70% increase in young neurons. C57BL/6 runners-for-food ran as much as voluntary runners, but they showed no enhancement of cell proliferation, a small increase in the number of young neurons and more pyknotic cells compared to controls. Taken together, these findings suggest that motivational aspects of running are critical determinants of the increased cell proliferation in C57BL/6 mice. In contrast, running has a smaller and contextindependent effect in house mice. The findings imply a difference in the regulation of AHN in inbred strains of mice and their wild-derived conspecifics.

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REFERENCES

Adlard PA, Perreau VM, Engesser-Cesar C, Cotman CW (The timecourse of induction of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus following voluntary exercise. Neurosci Lett 363:43-48.2004).

Allen DM, van Praag H, Ray J, Weaver Z, Winrow CJ, Carter TA, Braquet R, Harrington E, Ried T, Brown KD, Gage FH, Barlow C (Ataxia telangiectasia mutated is essential during adult neurogenesis. Genes Dev 15:554-566.2001).

Altman J (Are new neurons formed in the brains of adult mammals? Science 135:1127-1128.1962).

Altman J (Autoradiographic investigation of cell proliferation in the brains of rats and cats. Anat Rec 145:573-591.1963).

Altman J, Das GD (Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319-335.1965).

Amir S, Stewart J (Conditioned fear suppresses light-induced resetting of the circadian clock. Neuroscience 86:345-351.1998).

Amrein I, Dechmann DK, Winter Y, Lipp HP (Absent or low rate of adult neurogenesis in the hippocampus of bats (Chiroptera). PLoS ONE 2:e455.2007).

Amrein I, Slomianka L, Lipp HP (Granule cell number, cell death and cell proliferation in the dentate gyrus of wild-living rodents. Eur J Neurosci 20:3342-3350.2004a).

Amrein I, Slomianka L, Poletaeva, II, Bologova NV, Lipp HP (Marked species and age-dependent differences in cell proliferation and neurogenesis in the hippocampus of wild-living rodents. Hippocampus 14:1000-1010.2004b).

Andersen P, Morris R, Amaral DG, Bliss T, O`Keefe J (2006) The hippocampus book: Oxford University Press, USA.

Barker JM, Wojtowicz JM, Boonstra R (Where's my dinner? Adult neurogenesis in free-living food-storing rodents. Genes Brain Behav 4:89-98.2005).

Barnea A, Nottebohm F (Recruitment and replacement of hippocampal neurons in young and adult chickadees: an addition to the theory of hippocampal learning. Proc Natl Acad Sci U S A 93:714-718.1996).

Bartkowska K, Djavadian RL, Taylor JR, Turlejski K (Generation recruitment and death of brain cells throughout the life cycle of Sorex shrews (Lipotyphla). Eur J Neurosci 27:1710-1721.2008).

Bayer SA (Development of the hippocampal region in the rat. I. Neurogenesis examined with 3H-thymidine autoradiography. J Comp Neurol 190:87-114.1980).

Bedard A, Parent A (Evidence of newly generated neurons in the human olfactory bulb. Brain Res Dev Brain Res 151:159-168.2004).

Bednarczyk MR, Aumont A, Decary S, Bergeron R, Fernandes KJ (Prolonged voluntary wheel-running stimulates neural precursors in the hippocampus and forebrain of adult CD1 mice. Hippocampus 19:913-927.2009).

Ben Abdallah NM, Slomianka L, Vyssotski AL, Lipp HP (Early age-related changes in adult hippocampal neurogenesis in C57 mice. Neurobiol Aging.2010).

Bick-Sander A, Steiner B, Wolf SA, Babu H, Kempermann G (Running in pregnancy transiently increases postnatal hippocampal neurogenesis in the offspring. Proc Natl Acad Sci U S A 103:3852-3857.2006).

Blustein JE, McLaughlin M, Hoffman JR (Exercise effects stress-induced analgesia and spatial learning in rats. Physiol Behav 89:582-586.2006).

Brene S, Bjornebekk A, Aberg E, Mathe AA, Olson L, Werme M (Running is rewarding and antidepressive. Physiol Behav 92:136-140.2007).
Bruel-Jungerman E, Rampon C, Laroche S (Adult hippocampal neurogenesis, synaptic plasticity and memory: facts and hypotheses. Rev Neurosci 18:93-114.2007).
Bunk EC, Stelzer S, Hermann S, Schafers M, Schlatt S, Schwamborn JC (Cellular organization of adult neurogenesis in the Common Marmoset. Aging Cell 10:28-38.2011).
Cabib S, Puglisi-Allegra S, Oliverio A (Chronic stress enhances apomorphine-induced stereotyped behavior in mice: involvement of endogenous opioids. Brain Res 298:138-140.1984).
Caldwell CA, Whiten A (Testing for social learning and imitation in common marmosets, Callithrix jacchus, using an artificial fruit. Anim Cogn 7:77-85.2004).
Clark PJ, Kohman RA, Miller DS, Bhattacharya TK, Haferkamp EH, Rhodes JS (Adult hippocampal neurogenesis and c-Fos induction during escalation of voluntary wheel running in C57BL/6J mice. Behav Brain Res 213:246-252.2010).
Clelland CD, Choi M, Romberg C, Clemenson GD, Jr., Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH, Bussey TJ (A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325:210-213.2009).
Corkin S (What's new with the amnesic patient H.M.? Nat Rev Neurosci 3:153-160.2002).
Cotman CW, Berchtold NC, Christie LA (Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends Neurosci 30:464-472.2007).
Creer DJ, Romberg C, Saksida LM, van Praag H, Bussey TJ (Running enhances spatial pattern separation in mice. Proc Natl Acad Sci U S A 107:2367-2372.2006).
De Bono JP, Adlam D, Paterson DJ, Channon KM (Novel quantitative phenotypes of exercise training in mouse models. Am J Physiol Regul Integr Comp Physiol 290:926-934.2006).
Drapeau E, Mayo W, Aurousseau C, Le Moal M, Piazza PV, Abrous DN (Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. Proc Natl Acad Sci U S A 100:14385-14390.2003).
Dupret D, Revest JM, Koehl M, Ichas F, De Giorgi F, Costet P, Abrous DN, Piazza PV (Spatial relational memory requires hippocampal adult neurogenesis. PLoS ONE 3:e1959.2008).
Eisch AJ, Mandyam CD (Adult neurogenesis: can analysis of cell cycle proteins move us "Beyond BrdU"? Curr Pharm Biotechnol 8:147-165.2007).
Eisenstein SA, Holmes PV (Chronic and voluntary exercise enhances learning of conditioned place preference to morphine in rats. Pharmacol Biochem Behav 86:607-615.2007).
Epp JR, Barker JM, Galea LA (Running wild: neurogenesis in the hippocampus across the lifespan in wild and laboratory-bred Norway rats. Hippocampus 19:1040-1049.2009).
Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (Neurogenesis in the adult human hippocampus. Nat Med 4:1313-1317.1998).
Ferreira A, Lamarque S, Boyer P, Perez-Diaz F, Jouvent R, Cohen-Salmon C (Spontaneous appetence for wheel-running: a model of dependency on physical activity in rat. Eur Psychiatry 21:580-588.2006).
Festing MF (Wheel activity in 26 strains of mouse. Lab Anim 11:257-258.1977).
Fiore M, Amendola T, Triaca V, Alleva E, Aloe L (Fighting in the aged male mouse increases the expression of TrkA and TrkB in the subventricular zone and in the hippocampus. Behav Brain Res 157:351-362.2005).
Fordyce DE, Wehner JM (Physical activity enhances spatial learning performance with an associated alteration in hippocampal protein kinase C activity in C57BL/6 and DBA/2 mice. Brain Res 619:111-119.1993).

Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK, Berwald-Netter Y, Denoulet P, Chelly J (Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. Neuron 23:247-256.1999).

Gabrieli JD, Cohen NJ, Corkin S (The impaired learning of semantic knowledge following bilateral medial temporal-lobe resection. Brain Cogn 7:157-177.1988).

Garcia-Capdevila S, Portell-Cortes I, Torras-Garcia M, Coll-Andreu M, Costa-Miserachs D (Effects of long-term voluntary exercise on learning and memory processes: dependency of the task and level of exercise. Behav Brain Res 202:162-170.2009).

Garcia-Verdugo JM, Ferron S, Flames N, Collado L, Desfilis E, Font E (The proliferative ventricular zone in adult vertebrates: a comparative study using reptiles, birds, and mammals. Brain Res Bull 57:765-775.2002).

Gheusi G, Cremer H, McLean H, Chazal G, Vincent JD, Lledo PM (Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. Proc Natl Acad Sci U S A 97:1823-1828.2000).

Gibbs RA, Rogers J, Katze MG, Bumgarner R, Weinstock GM, Mardis ER, Remington KA, Strausberg RL, Venter JC, Wilson RK, Batzer MA, Bustamante CD, Eichler EE, Hahn MW, Hardison RC, Makova KD, Miller W, Milosavljevic A, Palermo RE, Siepel A, Sikela JM, Attaway T, Bell S, Bernard KE, Buhay CJ, Chandrabose MN, Dao M, Davis C, Delehaunty KD, Ding Y, Dinh HH, Dugan-Rocha S, Fulton LA, Gabisi RA, Garner TT, Godfrey J, Hawes AC, Hernandez J, Hines S, Holder M, Hume J, Jhangiani SN, Joshi V, Khan ZM, Kirkness EF, Cree A, Fowler RG, Lee S, Lewis LR, Li Z, Liu YS, Moore SM, Muzny D, Nazareth LV, Ngo DN, Okwuonu GO, Pai G, Parker D, Paul HA, Pfannkoch C, Pohl CS, Rogers YH, Ruiz SJ, Sabo A, Santibanez J, Schneider BW, Smith SM, Sodergren E, Svatek AF, Utterback TR, Vattathil S, Warren W, White CS, Chinwalla AT, Feng Y, Halpern AL, Hillier LW, Huang X, Minx P, Nelson JO, Pepin KH, Qin X, Sutton GG, Venter E, Walenz BP, Wallis JW, Worley KC, Yang SP, Jones SM, Marra MA, Rocchi M, Schein JE, Baertsch R, Clarke L, Csuros M, Glasscock J, Harris RA, Havlak P, Jackson AR, Jiang H, Liu Y, Messina DN, Shen Y, Song HX, Wylie T, Zhang L, Birney E, Han K, Konkel MK, Lee J, Smit AF, Ullmer B, Wang H, Xing J, Burhans R, Cheng Z, Karro JE, Ma J, Raney B, She X, Cox MJ, Demuth JP, Dumas LJ, Han SG, Hopkins J, Karimpour-Fard A, Kim YH, Pollack JR, Vinar T, Addo-Quaye C, Degenhardt J, Denby A, Hubisz MJ, Indap A, Kosiol C, Lahn BT, Lawson HA, Marklein A, Nielsen R, Vallender EJ, Clark AG, Ferguson B, Hernandez RD, Hirani K, Kehrer-Sawatzki H, Kolb J, Patil S, Pu LL, Ren Y, Smith DG, Wheeler DA, Schenck I, Ball EV, Chen R, Cooper DN, Giardine B, Hsu F, Kent WJ, Lesk A, Nelson DL, O'Brien W E, Prufer K, Stenson PD, Wallace JC, Ke H, Liu XM, Wang P, Xiang AP, Yang F, Barber GP, Haussler D, Karolchik D, Kern AD, Kuhn RM, Smith KE, Zwieg AS (Evolutionary and biomedical insights from the rhesus macaque genome. Science 316:222-234.2007).

Gobbo OL, O'Mara SM (Exercise, but not environmental enrichment, improves learning after kainic acid-induced hippocampal neurodegeneration in association with an increase in brain-derived neurotrophic factor. Behav Brain Res 159:21-26.2005).

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 2:260-265.1999a).

Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E (Hippocampal neurogenesis in adult Old World primates. Proc Natl Acad Sci U S A 96:5263-5267.1999b).

Gould E, Reeves AJ, Graziano MS, Gross CG (Neurogenesis in the neocortex of adult primates. Science 286:548-552.1999c).

Gould E, Tanapat P (Stress and hippocampal neurogenesis. Biol Psychiatry 46:1472-1479.1999).

Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E (Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci U S A 95:3168-3171.1998).

Greenwood BN, Foley TE, Day HE, Campisi J, Hammack SH, Campeau S, Maier SF, Fleshner M (Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. J Neurosci 23:2889-2898.2003).

Hauser T, Klaus F, Lipp HP, Amrein I (No effect of running and laboratory housing on adult hippocampal neurogenesis in wild caught long-tailed wood mouse. BMC Neurosci 10:43.2009).

Heine VM, Maslam S, Joels M, Lucassen PJ (Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus-pituitary-adrenal axis activation. Neurobiol Aging 25:361-375.2004).

Jabes A, Lavenex PB, Amaral DG, Lavenex P (Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus. Eur J Neurosci 31:273-285.2010).

Jaholkowski P, Kiryk A, Jedynak P, Ben Abdallah NM, Knapska E, Kowalczyk A, Piechal A, Blecharz-Klin K, Figiel I, Lioudyno V, Widy-Tyszkiewicz E, Wilczynski GM, Lipp HP, Kaczmarek L, Filipkowski RK (New hippocampal neurons are not obligatory for memory formation; cyclin D2 knockout mice with no adult brain neurogenesis show learning. Learn Mem 16:439-451.2009).

Jessberger S, Kempermann G (Adult-born hippocampal neurons mature into activity-dependent responsiveness. Eur J Neurosci 18:2707-2712.2003).

Johnson RA, Mitchell GS (Exercise-induced changes in hippocampal brain-derived neurotrophic factor and neurotrophin-3: effects of rat strain. Brain Res 983:108-114.2003).

Kaplan MS, Bell DH (Mitotic neuroblasts in the 9-day-old and 11-month-old rodent hippocampus. J Neurosci 4:1429-1441.1984).

Kaplan MS, Hinds JW (Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 197:1092-1094.1977).

Kempermann G, Gage FH (Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance, in the water maze task. Eur J Neurosci 16:129-136.2002a).

Kempermann G, Gage FH (Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. Brain Res Dev Brain Res 134:1-12.2002b).

Kempermann G, Kuhn HG, Gage FH (Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc Natl Acad Sci U S A 94:10409-10414.1997a).

Kempermann G, Kuhn HG, Gage FH (More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493-495.1997b).

Keuker JI, Luiten PG, Fuchs E (Preservation of hippocampal neuron numbers in aged rhesus monkeys. Neurobiol Aging 24:157-165.2003).

Kim YP, Kim HB, Jang MH, Lim BV, Kim YJ, Kim H, Kim SS, Kim EH, Kim CJ (Magnitude- and time-dependence of the effect of treadmill exercise on cell proliferation in the dentate gyrus of rats. Int J Sports Med 24:114-117.2003).

Kirn J, O'Loughlin B, Kasparian S, Nottebohm F (Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song. Proc Natl Acad Sci U S A 91:7844-7848.1994).

Kirn JR, Nottebohm F (Direct evidence for loss and replacement of projection neurons in adult canary brain. J Neurosci 13:1654-1663.1993).

Klaus F, Amrein I (Running in laboratory and wild rodents: Differences in context sensitivity and plasticity of hippocampal neurogenesis. Behav Brain Res: accepted manuscript).

Klaus F, Hauser T, Lindholm AK, Cameron HA, Slomianka L, Lipp HP, Amrein I (Different regulation of adult hippocampal neurogenesis in Western house mice (Mus musculus domesticus) and C57BL/6 mice. Behav Brain Res: reviewed manuscript).

Klaus F, Hauser T, Slomianka L, Lipp HP, Amrein I (A reward increases running-wheel performance without changing cell proliferation, neuronal differentiation or cell death in the dentate gyrus of C57BL/6 mice. Behav Brain Res 204:175-181.2009).

Kruska DC (On the evolutionary significance of encephalization in some eutherian mammals: effects of adaptive radiation, domestication, and feralization. Brain Behav Evol 65:73-108.2005).

Kuhn HG, Dickinson-Anson H, Gage FH (Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 16:2027-2033.1996).

Lambert MI, Van Zyl C, Jaunky R, Lambert EV, Noakes TD (Tests of running performance do not predict subsequent spontaneous running in rats. Physiol Behav 60:171-176.1996).

Lavenex P, Steele MA, Jacobs LF (The seasonal pattern of cell proliferation and neuron number in the dentate gyrus of wild adult eastern grey squirrels. Eur J Neurosci 12:643-648.2000).

Leasure JL, Decker L (Social isolation prevents exercise-induced proliferation of hippocampal progenitor cells in female rats. Hippocampus 19:907-912.2009).

Lerman I, Harrison BC, Freeman K, Hewett TE, Allen DL, Robbins J, Leinwand LA (Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains. J Appl Physiol 92:2245-2255.2002).

Leuner B, Glasper ER, Gould E (Sexual experience promotes adult neurogenesis in the hippocampus despite an initial elevation in stress hormones. PLoS One 5:e11597.2010).

Leuner B, Gould E, Shors TJ (Is there a link between adult neurogenesis and learning? Hippocampus 16:216-224.2006).

Lightfoot JT, Leamy L, Pomp D, Turner MJ, Fodor AA, Knab A, Bowen RS, Ferguson D, Moore-Harrison T, Hamilton A (Strain screen and haplotype association mapping of wheel running in inbred mouse strains. J Appl Physiol 109:623-634.2010).

Lightfoot JT, Turner MJ, Daves M, Vordermark A, Kleeberger SR (Genetic influence on daily wheel running activity level. Physiol Genomics 19:270-276.2004).

Lista I, Sorrentino G (Biological mechanisms of physical activity in preventing cognitive decline. Cellular Molec Neurobiol 30:493-503.2010).

Llorens-Martín MV, Rueda N, Tejeda GS, Florez J, Trejo JL, Martinez-Cue C (Effects of voluntary physical exercise on adult hippocampal neurogenesis and behavior of Ts65Dn mice, a model of down syndrom. Neurosci 171:1228-1240.2010).

Lonjon N, Prieto M, Haton H, Brochner CB, Bauchet L, Costalat V, Privat A, Gaviria M, Perrin FE (Minimum information about animal experiments: supplier is also important. J Neurosci Res 87:403-407.2009).

Maurer AP, Vanrhoads SR, Sutherland GR, Lipa P, McNaughton BL (Self-motion and the origin of differential spatial scaling along the septo-temporal axis of the hippocampus. Hippocampus 15:841-852.2005).

Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, Malapani C, Moore H, Hen R (Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. Nat Neurosci 9:729-731.2006).

Michaux JR, Chevret P, Filippucci MG, Macholan M (Phylogeny of the genus Apodemus with a special emphasis on the subgenus Sylvaemus using the nuclear IRBP gene and two mitochondrial markers: cytochrome b and 12S rRNA. Mol Phylogenet Evol 23:123-136.2002).

Ming GL, Song H (Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28:223-250.2005).

Mirescu C, Gould E (Stress and adult neurogenesis. Hippocampus 16:233-238.2006).

Namura S, Takada M, Kikuchi H, Mizuno N (Topographical organization of subicular neurons projecting to subcortical regions. Brain Res Bull 35:221-231.1994).

Naylor AS, Persson AI, Eriksson PS, Jonsdottir IH, Thorlin T (Extended voluntary running inhibits exercise-induced adult hippocampal progenitor proliferation in the spontaneously hypertensive rat. J Neurophysiol 93:2406-2414.2005).

O'Keefe J (Place units in the hippocampus of the freely moving rat. Exp Neurol 51:78-109.1976).

O'Keefe J, Dostrovsky J (The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res 34:171-175.1971).

Olson AK, Eadie BD, Ernst C, Christie BR (Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. Hippocampus 16:250-260.2006).

Ormerod BK, Lee TT, Galea LA (Estradiol enhances neurogenesis in the dentate gyri of adult male meadow voles by increasing the survival of young granule neurons. Neuroscience 128:645-654.2004).

Palm S, Roman E, Nylander I (Differences in voluntary ethanol consumption in Wistar rats from five different suppliers. Alcohol.2010).

Perfilieva E, Risedal A, Nyberg J, Johansson BB, Eriksson PS (Gender and strain influence on neurogenesis in dentate gyrus of young rats. J Cereb Blood Flow Metab 21:211-217.2001).

Ponti G, Peretto P, Bonfanti L (Genesis of neuronal and glial progenitors in the cerebellar cortex of peripuberal and adult rabbits. PLoS One 3:e2366.2008).

Ramón y Cajal S (1911) Histologie du Systéme Nerveux de l'Homme at des Vertébrés: A. Maloine, Paris.

Rhodes JS, van Praag H, Jeffrey S, Girard I, Mitchell GS, Garland T, Jr., Gage FH (Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. Behav Neurosci 117:1006-1016.2003).

Samorajski T, Delaney C, Durham L, Ordy JM, Johnson JA, Dunlap WP (Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice. Neurobiol Aging 6:17-24.1985).

Scoville WB, Milner B (Loss of recent memory after bilateral hippocampal lesions. 1957. J Neuropsychiatry Clin Neurosci 12:103-113.2000).

Siwak-Tapp CT, Head E, Muggenburg BA, Milgram NW, Cotman CW (Neurogenesis decreases with age in the canine hippocampus and correlates with cognitive function. Neurobiol Learn Mem 88:249-259.2007).

Slomianka L, Rungby J, West MJ, Danscher G, Andersen AH (Dose-dependent bimodal effect of low-level lead exposure on the developing hippocampal region of the rat: a volumetric study. Neurotoxicology 10:177-190.1989).

Snyder JS, Choe JS, Clifford MA, Jeurling SI, Hurley P, Brown A, Kamhi JF, Cameron HA (Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice. J Neurosci 29:14484-14495.2009a).

Snyder JS, Glover LR, Sanzone KM, Kamhi JF, Cameron HA (The effects of exercise and stress on the survival and maturation of adult-generated granule cells. Hippocampus 19:898-906.2009b).

Solberg LC, Horton TH, Turek FW (Circadian rhythms and depression: effects of exercise in an animal model. Am J Physiol 276:152-161.1999).

Squire LR, Alvarez P (Retrograde amnesia and memory consolidation: a neurobiological perspective. Curr Opin Neurobiol 5:169-177.1995).

Starborg M, Gell K, Brundell E, Hoog C (The murine Ki-67 cell proliferation antigen periphery of the mitotic chromosomes in a process essential for cell cycle progression. J Cell Sci 109 (Pt 1):143-153.1996).

Steppan SJ, Adkins RM, Spinks PQ, Hale C (Multigene phylogeny of the Old World mice, Murinae, reveals distinct geographic lineages and the declining utility of mitochondrial genes compared to nuclear genes. Molecular Phylogenetics and Evolution 37:370-388.2005).

Stranahan AM, Khalil D, Gould E (Social isolation delays the positive effects of running on adult neurogenesis. Nat Neurosci 9:526-533.2006).

Stranahan AM, Lee K, Mattson MP (Central mechanisms of HPA axis regulation by voluntary exercise. Neuromolecular Med 10:118-127.2008).

Tanapat P, Hastings NB, Reeves AJ, Gould E (Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J Neurosci 19:5792-5801.1999).

Thuret S, Toni N, Aigner S, Yeo GW, Gage FH (Hippocampus-dependent learning is associated with adult neurogenesis in MRL/MpJ mice. Hippocampus 19:658-669.2009).

Trejo JL, Llorens-Martin MV, Torres-Aleman I (The effects of exercise on spatial learning and anxiety-like behavior are mediated by an IGF-I-dependent mechanism related to hippocampal neurogenesis. Mol Cell Neurosci 37:402-411.2008).

van Praag H, Christie BR, Sejnowski TJ, Gage FH (Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 96:13427-13431.1999a).

van Praag H, Kempermann G, Gage FH (Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266-270.1999b).

van Praag H, Shubert T, Zhao C, Gage FH (Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 25:8680-8685.2005).

Vaynman S, Ying Z, Gomez-Pinilla F (Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 20:2580-2590.2004).

Werme M, Messer C, Olson L, Gilden L, Thoren P, Nestler EJ, Brene S (Delta FosB regulates wheel running. J Neurosci 22:8133-8138.2002).

West MJ, Slomianka L, Gundersen HJ (Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. Anat Rec 231:482-497.1991).

Wilbrecht L, Williams H, Gangadhar N, Nottebohm F (High levels of new neuron addition persist when the sensitive period for song learning is experimentally prolonged. J Neurosci 26:9135-9141.2006).

Winpenny E, Raineteau O (You are what you express: Glutamatergic neurogenesis in the adult SVZ. Cell Cycle 9.2010).

Wojtowicz JM, Askew ML, Winocur G (The effects of running and of inhibiting adult neurogenesis on learning and memory in rats. Eur J Neurosci 27:1494-1502.2008).

Yang H, Bell TA, Churchill GA, Pardo-Manuel de Villena F (On the subspecific origin of the laboratory mouse. Nat Genet 39:1100-1107.2007).

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